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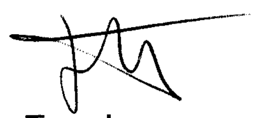

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ABSTRACT

Title of Thesis: "Effects of Environmental Conditions on Activity, Feeding, and Body Weight in Male and Female Adolescent Rats”

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Thesis directed by: Neil E. Grunberg, Ph.D.

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Excessive body weight, particularly in children, is a growing concern in the United States and around the world. Body weight is affected by feeding behavior and physical activity. Environmental factors affect feeding behaviors and physical activity; therefore, environment is an important influence on body weight. Three separate experiments examined the behavioral effects of environmental enrichments on feeding, activity, and body weight. For the first two experiments, subjects were 36 adolescent, male (Experiment I) and 36 adolescent female (Experiment II) Sprague-Dawley rats. Experiment III examined the behavioral effects of enrichment on 24 male and 24 female adolescent Sprague-Dawley rats. Responses to environmental enrichment included: body weight (BW), Body Mass Index score (BMI), Lee Index score (LI), consumption of standard rat chow, Oreo™ cookies, and Lays™ potato chips, and physical activity (PA) in the animal’s home cage (HCA) and in an open field (OF).

The major findings from these experiments were that: 1) environmental enrichment results in lower body weight, 2) environmental enrichment decreased

food consumption, especially the bland foods, 3) animals housed in environmental enrichment were less active in novel surroundings and were more active in their home cages compared to animals in non-enriched housing, and 4) males and females responded similarly to environmental enrichment with regard to body weight, feeding, and physical activity. These findings highlight the importance of the effects of housing conditions in animal research and suggest ways to help control body weight in animals and humans.

Effects of Environmental Conditions on Activity, Feeding, and Body Weight in Male and Female Adolescent Rats

by

Joshua L. Tomchesson

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"In order to attain the impossible, one must attempt the absurd."
- Miguel de Cervantes

In the beginning, the endeavor to become a skillful clinician and scientist appeared impossible. With desire, determination, the support of family and friends, and guidance from some extraordinary mentors, we attempted the absurd. What followed was an incredible experience unlike anything I ever imagined and the results of this expedition transcend what is written in this manuscript. This doctoral dissertation represents the successful completion of the first phase of my endeavor, a journey that does not seem impossible anymore. The successful completion of this phase of training would not have been possible without the knowledge, support, and guidance of many people.

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SECTION I – INTRODUCTION

Overview

Physical and social aspects of an environment can influence the biology and behavior of organisms. Biological changes in response to environmental manipulations can include changes in: brain structure (e.g., Rosenzweig, Bennett, & Diamond, 1972; Rosenzweig & Bennett, 1996; Mohammed et al., 2002), endogenous hormones such as corticosterone (Bhatnagar & Meaney, 1995; Mering Kaliste-Korhonen & Nevalainen, 2000; Belz, et. al., 2003), and body weight (Brown & Grunberg, 1995; O'Conner & Eikelboom, 2002; Hellemans, Benge, & Olmstead, 2004). Behavioral changes related to enriched environmental manipulations typically include improved performance on various tasks, such as maze performance and responses to novel situations. These improvements suggest that environmental enrichment improves learning, memory, and information processing (e.g., Hebb, 1947; Greenough & Juraska, 1979; Daniel, Roberts, & Dohanich, 1999; Varty et. al., 2000). The effects of environmental enrichment may be clinically relevant beyond learning, memory, or information processing changes because environmental changes also affect behaviors that are directly relevant to health. For example, environmental influences, such as cage size (Steyermark & Mueller, 2001), handling (Meaney, et al., 1992), type of available foods (Sclafani & Springer, 1976), and opportunities for activity (Lattanzio & Eikelboom, 2003), have been reported to affect food consumption and body weight of animals, two key factors of an organism's health.

This research project focused on the behavioral effects of environment on body weight because body weight is a current concern in our society. For example, approximately 65% of adults and 30% of children are at an increased risk for negative health related consequences associated with excessive body weight, such as premature death, diabetes mellitus, hypertension, cardiovascular disease, and certain kinds of cancers (Pi-Sunyer, 2004). This doctoral research included three experiments that were conducted to examine the extent to which: (1) environmental enrichment affects body weight, (2) environmental enrichment alters consumption of a variety of foods, (3) environmental enrichment affects physical activity, and (4) gender differences exist in these effects in adolescent rats. As background for this research, Section I reviews the relevant literature on environmental enrichment and body weight. Section II presents an overview for the work, the rationale for each independent and dependent variable, a description of the research design, and the data analytic strategy. Next, the hypotheses, methods, results, and a brief discussion for each of the three experiments are presented. Section III summarizes and discusses the findings of the project as a whole, including the limitations of this project and future directions for this research. Section IV includes relevant Tables, Figures, and References.

Enriched Environments

The concept of environmental enrichment has been used to improve the lives of animals and humans. Environmental enrichment refers to providing opportunities for organisms to thrive and excel. For captive animals, enrichment

includes providing physical objects and the opportunities for social interaction to create more naturalistic environments compared to barren cages (Shepherdson, 1992; Rosenzweig & Bennett, 1996). For humans, environmental enrichment includes providing individuals opportunities to be creative, learn, develop new skills, and promote growth (e.g., good nutrition, supportive and loving environments, performing music, painting, learning new languages, playing sports) (Diamond, 1999).

Environmental enrichment has long-lasting positive biological and behavioral consequences such as increasing brain size, and improving information processing and learning (e.g., Premack & Premack, 1963; Rosenzweig, Bennett, & Diamond, 1972; Rosenzweig & Bennett, 1996; Hellemans, Benge, Olmstead, 2004). In contrast, animals reared without physical or social stimulation exhibit less learning, decreased memory, and disrupted information processing compared to enrichment-reared animals (e.g., Greenough & Juraska, 1979; Woodcock & Richardson, 2000). Environmental changes also affect behaviors that are directly relevant to health, such as physical activity (e.g., Premack & Premack, 1963; Boakes & Dwyer, 1997; Lattanzio & Eikelboom; 2003) and food consumption (e.g., Brown & Grunberg, 1995; O'Conner & Eikelboom, 2002).

Research on environmental enrichment suggests that stimulating environments are important for healthy development and may influence the long-term health of the animals housed in these environments. Section I provides a discussion of environmental enrichment, the primary independent variable in the

proposed research project. A brief historical perspective provides the context to present the biological and behavioral effects of environmental enrichment and discuss the application of animal enrichment research findings to humans.

History of Enriched Environments

The notion of using the environment to affect future performance and behavior can be traced to early Chinese teachings. The concept of "intrauterine education" (i.e., educating a child during development within the womb) can be found in Chinese literature during the Ming Dynasty, around 1237 AD (Diamond & Hopson, 1999). Women were advised to behave favorably during pregnancy so that their offspring would be bright and live well. Favorable behavior included: sitting and walking in a dignified and sedate manner, maintaining a good temper with a mind at ease, not looking at evil happenings or ugly pictures (Diamond & Hopson, 1999). The popularity of intrauterine education is alive in our culture today, evidenced by products such as “Baby Bach” music CDs and concepts such as reading to the fetus through the mother’s belly to facilitate fetal intellectual development. The current conceptualization of environmental enrichment originated with Charles Darwin (1872).

Darwin (1872) reported that the brains of domestic rabbits were considerably smaller compared to the brains of wild rabbits. He argued that the reduced brain size of the domestic animals was a consequence of a deprived environment. Specifically, domesticated animals lived in inactive environments and did not exert their intelligence, instincts, or senses as much as animals did in

the wild. Empirical support for Darwin's observations and interpretation would not appear in the literature for decades.

In 1925, Robert Yerkes wrote about his experiences studying colonies of monkeys and apes in Cuba in his book Almost Human. He wrote that companionship and play were as important to a primate's health, comfort, and contentment as were physical environment and factors such as temperature, moisture, food, and drink. Further, he stated that no primate should be kept in isolation.

> "Undoubtedly, kindness to captive primates demands ample provision for amusement and entertainment as well as for exercise. The greatest possibility of improvement in our provision for captive primates lies in the invention and installation of apparatus which can be used for play or work." (Yerkes, 1925, p. 25)

Providing ample provisions for captive animals serves as a guiding principal for zoology and animal husbandry personnel throughout the world today.

In 1947, Donald Hebb observed that laboratory rats that he had taken home for his children to play with performed better on maze learning tasks when returned to the laboratory as compared to rats kept solely in the laboratory environment. He concluded that nerve cells in the brains of the rats had changed in response to the enriched and varied experiences outside the laboratory. Hebb hypothesized that the number of synaptic connections increased and that these structural changes resulted in functional (i.e., behavioral) modifications. These changes were believed to reflect new learning. Remarkably, Hebb's report,

which was consistent with Darwin's (1872) observation, did not generate research for almost 20 years.

Mark Rosenzweig and his colleagues at the University of California, Berkeley, introduced the classic paradigm of enriched environments in 1966. In Rosenzweig's (1966) enrichment, animals were provided both social and physical stimulation. Animals were housed in groups to provide opportunities for social interaction (i.e., social enrichment). Physical stimulation (i.e., physical enrichment) involved placing objects in the animals' cages to allow tactile stimulation and physical activity (Rosenzweig & Bennett, 1996; Woodcock & Richardson, 2000). Enriched environments are distinguished from non-enriched environments by the amount of stimulation and activity available in the environment. The standard non-enriched environment limits the physical and social enrichment by housing the animals individually without objects (Varty, Paulus, Braff, & Geyer, 2000). Most current environmental enrichment studies include both social and physical enrichment components (e.g., Pham et al., 1999; Passineau, Green, & Detrich, 2001; Tomchesson, 2004).

There is no one method of environmental enrichment in the research literature. There are many different ways to manipulate and conceptualize environmental enrichment in animals including: neonatal handling (Meaney, Aitken, Sharma, & Viau, 1992), pretest handling (Schmitt & Hiemke, 1997), social enrichment (Renner & Rosenzweig, 1986; Varty et al., 2000), physical enrichment (Renner & Rosenzweig, 1986; Varty et al., 2000), and incorporation of natural environmental objects (Schrijver et al., 2002). Enriched environments

also vary in the amount of time animals are exposed to enrichment ranging from 12 days (Passineau, Green, & Detrich, 2001; Elliott & Grunberg, 2005) to a year (Ickes et al., 2000). The most common enriched environments in animal research house 3 to12 subjects per cage filled with toys and objects (e.g., pieces of wood, plastic bones, exercise wheels, balls, tunnels) for a minimum of 2 - 4 weeks.

Effects of Enriched Environments

Enriched housing environments have different biological and behavioral consequences when compared to non-enriched housing. Although this research project focused on the behavioral consequences, biological consequences are also important to this research. This section briefly reviews biological and behavioral consequences of environmental enrichment (EE).

Biological Effects of Enrichment

Although human beings are born with 100 billion neurons that are surrounded by over one trillion glial cells that protect and nourish these neurons, the pattern of "wiring" necessary for communication between the cells is not yet stabilized at birth (Nash, 1997; Joseph, 1999). For example, the number of synapses in one layer of the visual cortex increases from approximately 2,500 connections at birth to as many as 18,000 connections only six months later (Kliem et al., 1998). The environment may contribute to the exact wiring that occurs because altering environments appears to alter brain cytoarchitecture (Mohammed et al., 1993; Rosenzweig, 1996; Diamond, 2001).

Animal experiments reveal that physical and social stimulation (i.e., environmental enrichment) evoke the same cascade of neurochemical events that cause plasticity alterations in the human brain (Rosenzweig & Bennett, 1996). Stimulating environmental conditions (i.e., enriched environments) significantly influence brain development. These influences include: increased size and weight of the cortex, increased neuron sizes and dendritic branching, increased synapse formation, and elevated protein levels (Rosenzweig, Bennett, & Diamond, 1972; Mohammed et al., 2002). Diamond (1991) reported that laboratory rats housed in enriched environments could have up to 25 percent more neurons in their brains when compared to rats housed in non-enriched environments. In addition to biological changes in animals, behavioral changes also have been reported.

Behavioral Effects of Enrichment

In addition to the increased number of neurons in the brain, rats reared in an enriched environment exhibit more complex behaviors than rats reared in non-enriched environments (Haywood & Tapp, 1966; Mohammad et al., 1993; Pham et al., 1999; Kobayashi, Ohashi, & Ando, 2002). Environmental enrichment can significantly improve the cognitive functioning of animals. This improvement is inferred from the animal's performance on behavioral tasks of attention, memory, and learning compared to animals reared in standard non-enriched environments. For example, early social isolation leads to an interruption of attentional processing in rats as measured by acoustic startle reflex (Robbins, 1996). Rats reared with environmental enrichment are better

able to identify relevant environmental cues that decrease the magnitude of their reflexive responses to loud noises. Also, rats deprived of social contact post-weaning (i.e., when social play normally develops) have an impaired ability to process information as measured by pre-pulse inhibition (PPI) of the acoustic startle reflex, believed to index an innate sensorimotor “gating” mechanism that underlies the organism’s ability to select relevant stimuli from the environment while screening out irrelevant information (Swerdlow, Caine, Braff, & Geyer, 1992).

Superior learning and memory task performance by rats reared in enriched environments is well documented (Greenough & Juraska, 1979). Woodcock and Richardson (2000) reported superior information processing and working memory for rats raised in enriched environments compared to rats raised in non-enriched environments. Rats reared in enriched environments were better able to discriminate between two types of cages: a conditioning cage used to train the animals and a similar cage that looked like the conditioning cage but was distinctly different. The Morris water maze and the radial maze tasks are widely used measures of rodent learning and spatial memory. When compared to rats housed in non-enrichment conditions, the rats housed in enriched conditions perform significantly better in the Morris water maze task (Daniel, Roberts, & Dohanich, 1999; Williams, Luo, Ward, Redd, & Gibson, 2001; Elliott, 2004) and the radial maze (Einon, 1980). Enriched housing environments also result in more rapid decreases in locomotor activity in novel environments, indicating faster learning and adaptation to the new environment (Elliott, 2004). Similarly,

rats reared in enriched environments display quicker adaptation of the acoustic startle response than rats reared in non-enriched environments (Swerdlow, Caine, Braff, & Geyer, 1992; Wilkinson et al., 1994; Elliott, 2004). These findings suggest that environmental enrichment enhances the animal's ability to process information, thereby improving the overall cognitive abilities of rats housed in enriched environments compared to rats housed in non-enriched environments.

The benefits of environmental enrichment are not limited to rats and have been reported in other animals. Two examples of the diverse species that have benefited from environmental enrichment are seals and pigs. Grindrod and Cleaver (2001) reported that incorporating novel toys, such as rubber balls, and opportunities to forage for food reduced captive seals' stereotypic circling behavior. Stereotypic behaviors (e.g., circling or pacing) are abnormal repetitive behaviors commonly thought to represent anxiety or stress in animals (Grindrod & Cleaver, 2001). Consequently, the addition of toys and opportunities to work for food reduced the emotional response of the seals to their environment. In addition to seals, pigs also benefit significantly from environmental enrichment. Enrichment for pigs consists of social housing, straw, and toys such as cloth strips, chains, dirt, and ropes (Grandin, Curtis, & Greenough, 1983; Grandin, Curtis, & Taylor, 1987). Pigs housed in barren environments will use penmates as substitutes for other objects resulting in harmful social behaviors (Buré, 1981; Burbidge et al., 1994). However, enlarged housing spaces and partial stalls used to keep the animals from seeing each other, decreases aggression, reduces cortisol concentrations, and increases immunological responsiveness (Andersen

et al., 1999). Wemelsfelder, Haskell, Mendl, Calvert, and Lawrence (2000) suggest that enriching the environment of pigs counteracts frustration and boredom that typically results from chronic under-stimulation. Additionally, pigs reared in enriched environments exhibit more diverse behaviors than pigs reared in non-enriched environments (Whittaker et. al., 1998; Wemelsfelder et al., 2000).

Enriched environments, characterized by the presence of physical objects and the opportunity for social interaction, have been shown to enhance healthy brain development and provide marked improvements in performance. Research has demonstrated that enrichment leads to improved performance in learning tasks and a significant reduction of emotional responses to the environment. In contrast, non-enriched environments or reduced sensory stimulation, such as social isolation and the lack of physical objects, results in performance deficits in learning tasks and expressed hyper-emotionality. The benefit of environmental enrichment for animals is important in the context of captive environments; however, the power of environmental enrichment is in its application to humans.

Relevance of Enriched Environments to the Human Condition

Developed from animal research, the concept of environmental enrichment has been used effectively by humans. In the late 1960's, Mark Rosenzweig, Marion Diamond, and their colleagues reported neurological changes within the animal brain in response to environmental enrichment. These changes include increased size and weight of the cortex, increased neuron sizes and dendritic branching, and increased synapse formation. Based on these reports and

findings that environmental enrichment improved performance on behavioral learning tasks, the inference that the reported neurological changes accounted for the improved performance was extrapolated to humans. Consequently, the animal research on environmental enrichment inspired human enrichment programs to attempt to improve learning, memory, and information processing.

The application of environmental enrichment to help humans proved to be a valuable exercise. Research has shown that environmental stimulation appears to be necessary for healthy brain development and may affect behaviors later in life. For example, children raised in impoverished environments exhibit impairments in cognitive and behavioral functioning, whereas children raised in highly stimulating or enriched environments exhibit enhanced behavioral and cognitive outcomes (Kaler & Freeman, 1994; Joseph, 1999). Haywood and Tapp (1966) suggested that limited intelligence and social functioning often accompany "unstimulating" childhood conditions. Experimental enrichment programs have been able to offset some of the cognitive and social deficits created by unstimulating conditions. In a recent study, an environmentally-based intervention showed promising results in Portuguese elementary school children who were previously exposed to adverse environmental factors (e.g., malnutrition, familial distress, and low family income). Children were placed together in a boarding house (i.e., social enrichment) and were taught using a learning program that included psychomotor exercises, painting, singing, computer training, and language skills (English and Spanish). Placing the children in the social housing condition and enriching their instruction significantly

improved the children's school attendance and classroom behavior. More importantly, this better school attendance was correlated with significant improvements in the students' cognitive performance (Macedo, Andreucci, & Montelli, 2004). Therefore, the enrichment program resulted in improvements in behavior and academic performance.

One of the most notable applications of environmental enrichment is the introduction of early education programs, such as "Head Start." "Head Start" is a comprehensive preschool program that began in 1965. It was designed to meet the emotional, social, health, nutritional, educational, and psychological needs of children, ages 3 to 5, from low-income families. In general, the research on the efficacy of such early education programs indicates that participating in these programs improves cognitive abilities and skill attainment for children exposed to these enrichment programs, compared with children who were not exposed to early educational programs, for up to several years after the end of the program (Behrmen et al., 1995). Reynolds and colleagues (2001) completed a 15-year follow-up study that examined specific behavioral consequences of early education and reported significant differences between children who attended "Head Start" programs and those who did not. Children in "Head Start" programs had lower high school drop-out rates (46.7% vs. 55%), a lower rate and number of juvenile arrests (16.9% vs. 25.1%), lower rates of children retained in their current academic grade (23% vs. 38.4%), and lower rates of required special education programs (15.4% vs. 21.3%) (Reynolds et al., 2001).

Additional research suggests that environmental enrichment may produce behavioral changes in children, such as increased impulse control, more systematic planning and organization, and more academic risk-taking in the classroom (Wilson, 1996). There are consistent data suggesting that students who take music and art classes acquire higher SAT and College Board scores (The College Board, 1996-2004). These higher scores reflect improved cognitive abilities such as verbal skills, math, and abstract reasoning (Graziano, Peterson, & Shaw, 1999). Further, a positive correlation between the length of time spent studying the arts and improved SAT scores has been reported. Individuals who spent four or more years studying music and art scored an average of 59 points higher on verbal and 44 points higher on math portions of the SAT compared to students with no coursework or experience in the arts (MENC, Profiles of SAT and Achievement Test Takers, 1995).

It is evident that environmental enrichment can positively affect the biology and behavior of animals. More importantly, the concept of environmental enrichment first investigated in animals has influenced programs designed to enhance human behavior and development. The research suggests that humans are directly influenced by their environment and have benefited from environmental enrichment. Therefore, animal models are a valuable means to investigate the effects of environmental enrichment to inform the human condition.

Body Weight

Body weight is a quantitative measure that can be used to track developmental growth and to index health. Excessive body weight, particularly among children, is a growing health concern in America. The following section describes body weight and excessive body weight. The measurement of body weight, the epidemiology, the consequences associated with excessive body weight, and body weight regulation are discussed.

Description and Measurement

Weight is the force of gravity on a mass, expressed as a number representing the mass' heaviness (Hewitt, 1997). Accordingly, body weight is the force of gravity on an individual's body mass. Body weight is the net result of daily energy intake and energy expenditure and typically increases as an organism grows. When the daily intake of energy exceeds the amount of daily energy expended, body weight increases (Boon, Visser, & Daan, 1997; Warwick, Synowski, & Bell, 2002). Excess energy is stored in the body as fat. As energy intake exceeds the amount of energy expended, the amount of fat within the body increases and body weight increases. The measurement of body fat is important because the presence of excess body fat increases risks for premature death or other negative health consequences (e.g., diabetes or cardiovascular disease).

Body weight varies greatly and depends on an individual's body composition. Consequently, defining the "ideal," "normal," or "excessive" body for a given individual can be difficult. Despite this fact, desirable weight

standards are typically determined in two ways. The first method is to create a weight table based on the actual heights and weights collected from a representative or sample population. These types of normative tables have been developed by insurance companies (e.g., Metropolitan Life Insurance Company) and by the National Center for Health Statistics (NCHS) (NRC, 1989; CDC, 2002) for many years. The NCHS identifies overweight individuals as persons whose weights are at or above the 85th percentile of weight for height. Those individuals with weights represented by the top 5th percentile are considered severely overweight or obese. There are two major drawbacks to using this normative sample approach. First, the desirable weight standards change as the weight distribution of the population changes. Second, the underlying assumption is that the "average" weight is a healthy or preferred weight and this assumption may be inaccurate (Andres et al., 1985). Health-care providers and investigators studying body weight prefer the Body Mass Index (BMI) to determine one's desirable body weight (NIH, 1995). BMI is a mathematical formula (a ratio of weight to height^2) that is highly correlated with body fat (Korner & Aronne, 2003). It is expressed as weight in kilograms divided by height in meters squared (BMI = kg/m ^{2}). BMI is preferred because it correlates highly with body fat, adjusts for height, and uses "cut-off" values associated with health risks.

The definition of a "normal" or "healthy" BMI was recently changed in 2000. Federal agencies have made a transition to define overweight as a BMI using the recommendations in the current edition of Dietary Guidelines for Americans

(2000) (Kuczmarski & Flegal, 2000). Consistent with internationally recommended BMI cut-offs, a BMI of < 25 kg/m^2 is considered normal, overweight is reflected by a BMI of 25 – 29.9 kg/m^2, and obesity is identified as a BMI of > 29.9 kg/m^2 (NCHS, Public Health Service, 2000; Korner & Aronne, 2003). Prior to this change there was an inconsistent use of BMI cut-offs based on references and standards that affect the numbers of Americans classified as overweight and obese. Kuczmarski and Flegal (2000) reported that when BMI cut-offs of ≥ 27.8 for men and ≥ 27.3 for women were applied to the National Health and Nutrition Examination Survey (NHANES) III data, the prevalence of overweight among adults aged ≥ 20 years was 33.3% for men and 36.4% for women. In contrast, at a BMI ≥ 25.0, the prevalence was 59.4% for men and 50.7% for women. By changing the overweight cut-offs, the estimated number of overweight adults increased from 61.7 million (BMI ≥ 27.8 and 27.3) to 97.1 million (BMI ≥ 25.0), representing a difference of 35.4 million overweight adults (Kuczmarski & Flegal, 2000). Regardless of this change in categorization, excessive weight continues to increase at an alarming rate in the United States.

In addition to body weight, body fat can be inferred from a number of measures. The most accurate means to measure body fat is through hydrodensitometry or hydrostatic weighing (HW); i.e., measuring body density via water displacement (Levenhagen et. al., 1999). This method is cumbersome and time consuming, so easier, more efficient measures are often used. Body fat can be estimated by skinfold measures, waist-to-hip circumference ratios, or techniques such as ultrasound, computer tomography (CT), magnetic resonance

imaging (MRI), bioelectrical impedance (BAI), or Body Mass Index (BMI) (NIH, 1995). The most widely used measure to estimate body fat is BMI.

Excessive Body Weight, Description, Epidemiology, and Consequences

Excessive body weight is described using the terms overweight and obesity. Overweight refers to increased body weight in relation to height, when compared to some standard of acceptable or desirable weight (NIH, 1998). Overweight may be the result of increases in body fat or it may be a result of an increase in lean muscle mass. For example, professional athletes may be lean and muscular, with little body fat, yet they may weigh more than others of the same height. Obesity is defined as an excessively high amount of body fat or adipose tissue in relation to lean body mass (NIH, 1998).

A high prevalence of overweight and obesity is of great public health concern. The prevalence of obesity among adults has doubled in the past two decades (Flegal et al., 2002; Hedley et al., 2004). Over 65 percent of Americans are estimated to be overweight or obese, defined as a BMI > 25 kg/m^2. According to recent data (1999-2002), approximately 31% of adults $\geq$ 20 years of age (over 60 million people) had BMI $\geq$ 30 kg/m^2 compared to 23% in 1994 (Flegal et al., 2002). The problem of excessive weight is not limited to the United States. An estimated 1.7 billion people worldwide are overweight or obese (National Institutes of Health, 2004).

The number of children that are overweight or obese is reaching epidemic proportions in the United States. The number of overweight children has doubled since 1980 (7% in 1980 to 16.5% in 1999–2002). The number of overweight

adolescents has tripled in that same time period (5% in 1980 to 16% in 1999 – 2002) (Hedley et al., 2004). An additional 15 percent of children are considered to be at risk for being overweight (Hedley et al., 2004). Excessive body weight in children is particularly concerning because the risks of negative health consequences increases the longer one has excessive body weight.

This increase in excessive body weight has severe negative health consequences. Excessive body weight can result in elevated risks for premature death and for many serious medical conditions, including: diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, stroke, gall bladder disease, respiratory dysfunction, gout, osteoarthritis, and certain kinds of cancers (Pi-Sunyer, 2004). Two important factors associated with the risk of developing several chronic diseases are total body fat and the distribution of the fat on the abdomen and trunk or peripherally on the arms and legs (NIH, 1995). It is ironic that obesity is increasing in the United States while more people are dieting than ever before and spending $30 billion to $50 billion yearly on weight-reduction products (including diet foods and soft drinks, artificial sweeteners, and diet books) and services (e.g., fitness clubs and weight-loss programs) (Kassirer & Angell, 1998). According to the recent figures, medical expenses for overweight and obesity accounted for 9.1% of total U.S. medical expenditures in 1998 (Wolf, 1998; Finkelstein, Fiebelkorn, & Wang, 2004) and was estimated to cost $117 billion in the U.S. for the year 2000 (combination of direct health care costs plus indirect costs, such as lost wages caused by illness) (U.S. Department of Health and Human Services, 2001). It is estimated that Medicare and Medicaid

programs pay about $39 billion a year for treatment of medical conditions related to excessive weight gain (e.g., diabetes, cardiovascular disease, cancer) (Finkelstein, Fiebelkorn, & Wang, 2003). Clearly, excessive body weight is a critical problem in America.

Body Weight Regulation

There are four interrelated factors that lead to body weight changes: metabolic activity (Bray & York, 1979; Westertep-Plantenga, 2004), genetics (Meyer & Stunkard, 1993), daily energy intake (Sclafani & Springer, 1976; Sclafani & Gorman, 1977; Warwick, Synowski, & Bell, 2002), and daily energy expenditure (Premack & Premack, 1963; Lattanzio & Eikelboom, 2003). Animal studies provide clear evidence that metabolic activity and genetics are important factors in excessive weight gain (Bray & York, 1979). The most clear-cut examples are animals with recessively inherited forms of obesity that had greater weight gain and fat deposits compared to their lean littermates, despite precise paired feeding (Cox & Powley, 1981). Further, biological defects (e.g., hypothalamic lesions) and the observation that obesity runs in families from twin and adoption studies also support the contention that metabolic activity and genetics are important factors to consider (Meyer & Stunkard, 1993). However, metabolism and genetics explain about half of the variance in body weight, whereas energy intake and intake account for the other half (Leamy et. al., 2005).

This doctoral research project focused on the behavioral effects of the environment on body weight in rats. As such, energy intake and physical activity

are most relevant to the project and are discussed in more detail. The following two parts of this section discuss the relationship of each variable (feeding and physical activity) to body weight.

Energy Intake: Body Weight and Food Consumption

Changes in food intake can produce changes in body weight (Sclafani & Springer, 1976; Forbes, 1987; Iossa, Lionetti, Mollica, Barletta, & Liverini, 1999). An increase in energy intake by increasing calories can lead to weight gain. An increase in calories can be accomplished in two ways: (1) by increasing the amount of food provided or portion size (Sclafani & Springer, 1976; Schachter & Rodin, 1974), or (2) by consuming foods of high caloric density (e.g., nuts, fast foods, potato chips, or cookies) (Iossa, Lionetti, Mollica, Barletta, & Liverini, 1999; Warwick, Synowski, & Bell, 2002). There is a linear relationship between the amount of increased food intake and change in body weight that allows weight gain to be predicted from the amount of excess caloric ingestion (Forbes, 1987).

In a classic study of starvation in humans, Keys and colleagues (1950) reported that reduced-calorie diets were associated with weight loss in normal, healthy volunteers. Additionally, calorie restriction in obese subjects reduced body weight and fat stores (Forbes, 1987). In animals, increasing total caloric intake causes them to increase body weight. There are several ways of increasing voluntary food intake in animals, including the provision of more palatable diets (Sclafani and Springer, 1976), the administration of calorically dense drinking solutions (Warwick, Synowski, & Bell, 2002; Sclafani 2004),

switching diets frequently (Collier, 1985), and increasing the fat content of the diet (Warwick, Synowski, & Bell, 2002). From both human and animal research, the evidence suggests that increases in body weight result from increased daily energy intake that exceeds daily energy expenditure through the amount and types of foods that are eaten.

The control of energy intake is dependent upon satiety and two related concepts, hunger and appetite. Satiety is the state of being full to or beyond capacity (Graaf et al., 2004). Satiety is controlled by hunger and appetite, responses to internal and external signals that are related to energy balance (Graaf et al., 2004). Hunger is the physiological need for food and appetite is the psychological motivation for food intake (Lasagna, 1988; Andersen, 1996). Hunger and appetite control rely on peripheral physiology and metabolic interactions in the brain. The regulation of body weight is a homeostatic system that involves a negative feedback loop between intake and compensated factors (De Castro & Plunkett, 2002). Examples of compensated factors include plasma levels of blood glucose, free fatty acids, body weight, Central Nervous System (CNS) insulin, and hypothalamic neuropeptide-Y (NPY). Circulating inhibitory signals, produced in proportion to fat mass, act on the brain to reduce or increase hunger, which in turn increases or decreases appetite (Connan & Stanley, 2003). In addition, inhibitory signals are generated in response to individual meals, which alter energy intake. Fulton et al. (2000) reported that leptin acts to attenuate the reward produced by self-stimulation in some areas of the brain but to enhance these effects in other areas. Leptin acts on the circuits influencing

appetite and energy available for expenditure and also acts on the motivation to eat (Connan & Stanley, 2003). Therefore, appetite can control energy intake and can mediate weight changes.

Appetite control is accomplished at three levels: (1) patterns of eating behaviors, (2) intermediary physiological mechanisms, and (3) brain pathways (De Castro & Plunkett, 2002). Patterns of eating behavior range on a continuum from early to late control. Early control is accomplished via sensory information (e.g., an empty plate) followed by cognitive control (e.g., complex information processing regarding the current context). Later control is accomplished through post-ingestive and post-absorbative behaviors (e.g., getting up from the table immediately after eating or sleeping after digestion). Intermediary physiological mechanisms involve chemical signaling via several circulating peptides, including leptin, insulin, cholecystokinin (CCK), glucagon, neurotensin, and somatosatin (Connan & Stanley, 2003). As a meal is consumed, the ingested food interacts with these intermediary mechanism receptors leading to release of peptides.

The last level of control is accomplished as the circulating peptides enter and activate central circuits within the brain. The hypothalamus is the primary center for appetite control. Within the hypothalamus, the arcuate nucleus (ARC), paraventricular nucleus (PVN), and the lateral hypothalamus are critical sites for the action of peripheral signals (Connan & Stanley, 2003). Leptin, a signal of fat mass, and insulin, the hormone responsible for the breakdown of glucose, are the primary peripheral signals of energy homeostasis. Both of these peptides are secreted in proportion to adipose tissue and act directly on the ARC and PVN of

the hypothalamus (Connan & Stanley, 2003). The ARC is implicated as essential in regulation of the energy balance because its destruction produces obesity (Olney, 1969). The PVN is implicated in weight control, its destruction results in weight gain, and electrical stimulation of the PVN decreases appetite (Weingarten et al., 1985). In addition, dopamine and the brain's reward system are implicated in food consumption. Specifically, dopamine release is decreased in association with increases in obtaining food and dopamine release is enhanced by food deprivation (Berridge & Robinson, 2003). ***Energy Expenditure: Body Weight and Activity***

Physical activity is important for weight control and affects distribution of body fat (Cortwright, Chandler, Lemon, & DiCarlo, 1997). By using energy and maintaining muscle mass, physical activity is a useful and effective adjunct to dietary management. Increased fat mass and obesity occur when energy intake exceeds total daily energy expenditure for a prolonged period (Leibel, Rosenbaum, & Hirsch 1995). One way total energy intake can exceed total daily expenditure is through reduced physical activity.

Physical activity is defined as bodily movement produced by the contraction of skeletal muscle that increases energy expenditure above the basal level. Physical activity can be categorized in various ways, including the type, intensity, and the purpose of the activity. Common activity types include occupational, household, leisure time, or transportation. An example of physical activity classified by intensity is high, medium, or low impact aerobics. To categorize the purpose of physical activity, leisure time activity can be subdivided

into categories such as competitive sports, recreational activities (e.g., hiking, cycling), and exercise training (Surgeon General's Report on Physical Activity and Health, 1996; USDHHS, 2001). An important distinction to make is between physical activity and exercise because these terms are often misused interchangeably. Physical activity differs from exercise in that exercise is planned, structured, and repetitive physical activity targeted to improve or maintain one or more components of physical fitness (Caspersen, Powell, & Christensen 1985).

The distinction between physical activity and exercise is relevant to the current investigations for two reasons. First, this project monitored physical activity as opposed to a forced exercise program (e.g., swimming or running wheel) in rats. Physical activity was used to examine what naturally happens to animal activity, food consumption, and body weight in response to various housing conditions. Previous research clearly demonstrates that animals forced to exercise lose weight (Premack & Premack, 1963; Boakes & Dwyer, 1997; Mueller, Herman, & Eikelboom, 1999; Lattanzio & Eikelboom, 2003) and the purpose of the project was to examine the animals' responses to environmental conditions. Second, exercise is one type of physical activity with the purpose of improving physical fitness and the animals' physical fitness was not being addressed in this study.

Increased physical activity in the absence of increased caloric intake leads to a decrease in body weight and a change in body composition in animals. The amount of caloric expenditure is proportional to these effects and this relationship

is more pronounced for male compared to female rats (Keesey & Powley, 1986). Rats reared using two different photoperiod schedules, one with an 18-hour sleep cycle and one with a shorter sleep cycle of 6 hours, showed differences in energy expenditure. The animals exposed to the 18-hour sleep cycle (i.e., less activity period) had lower daily energy expenditure, a higher rate of weight gain, less lean body mass, and similar energy intakes compared to the animals in a 6-hour sleep cycle (Boon, Visser, Daan, 1997). These findings support the importance of energy expenditure as a critical variable to weight gain because energy intake was similar in these studies. Animals given opportunities to exercise (i.e., a running wheel) consistently weigh less compared with controls not given opportunities to exercise (Premack & Premack, 1963; Boakes & Dwyer, 1997; Mueller, Herman, & Eikelboom, 1999; Lattanzio & Eikelboom, 2003). These findings indicate that physical activity is an important variable in animal body weight and that the environment the animal is in affects physical activity.

Together, the data on energy intake regarding food consumption, and the data on energy expenditure regarding physical activity indicate that the environment can exert a powerful effect on body weight. The data on food consumption indicate that manipulation of the amount and type of available foods can significantly affect body weight. The data regarding physical activity clearly indicate that increasing physical activity so that energy expenditure exceeds energy intake leads to weight loss. Varying components of the environment such as available foods, lighting, and availability for physical activity are likely to be important influences on body weight. For example, access to more physical

activity via a larger cage or the availability of different types of foods in these cages may be important environmental factors to consider in body weight changes.

Environmental Enrichment and Body Weight

Few studies have reported the effects of environmental enrichment on body weight. Environmental enrichment has been reported to decrease body weight in adult rats (Sclafani & Springer, 1976) and adolescent rats (Hellemens, Benge, & Olmstead, 2004; Tomchesson, 2004). However, enrichments effects on body weight have not been studied extensively. This section describes three experiments relevant to this research project, presents potential mechanisms of action of the effects of enrichment on body weight, and identifies several limitations of research reporting findings relevant to environmental enrichment effects on body weight.

Three studies provide the foundation for this research project: Sclafani and Springer (1976), Hellemens, Benge, and Olmstead (2004), and Tomchesson (2004). Sclafani and Springer (1976) studied weight gain in adult female, Sprague-Dawley rats given access to a variety of foods. Sixteen animals were placed in one of three conditions for 65 days: an isolation condition (where animals were individually housed in standard wire mesh cages, 15.5 cm X 25 cm X 17.5 cm), an active condition (where animals were housed in a small wire mesh cage attached to a running wheel), and an environmentally-enriched condition called a “complex environment.” In the “complex environment” eight littermates were housed together in two large, three-level, wire mesh cages (45

cm X 47.5 cm X 67.5 cm) that included wooden, metal, and plastic objects in the upper two levels.

Regardless of their housing condition, animals provided with a “cafeteria diet” (cookies, potato chips, salami, marshmallows, etc.) gained more weight than did the rats that were provided standard chow. The animals with access to the activity wheel (activity condition) gained less weight than did the rats in the isolated or complex environments (Sclafani & Springer, 1976). Animals in the complex environment gained as much weight on the “cafeteria diet” as did the animals in the isolated condition, indicating that the type of foods available and that opportunity for physical activity are important factors in the animals’ weight gain. The authors suggested that a larger and more complex environment might limit the development of obesity in these adult rats but did not elaborate on the mechanisms (e.g., increasing activity or decreasing food intake). Because the amount of activity is a critical variable in determining body weight, it is possible that providing larger cage sizes can allow greater availability for physical activity and may result in lower body weights by increasing energy expenditures.

Sclafani and Springer (1976) also reported that the activity group with access to a running wheel weighed significantly less compared to the other groups when all animals had access to bland foods. Access to a running wheel also attenuated some of the weight gain in the animals fed the cafeteria diet. The findings from this study suggest that monitoring physical activity and controlling the types of available foods should be investigated together. This study indicates that opportunities for activity help to control body weight in adult

female rats. This experiment did not examine adolescent or male rats and did not measure home cage activity.

Hellemans, Benge, and Olmstead (2004) examined body weight in male, Long-Evans rats that were fed standard rat chow. The adolescent rats (21 day old upon arrival) were placed into three different housing conditions: isolated, standard housing, and enriched (consisting of 12 litter mates and toys) for 12 weeks. Isolation reared animals were housed singly in cages without physical stimuli (40 cm X 10 cm X 10 cm). Rats reared in the standard condition were housed in pairs with no physical stimuli (40 cm X 10 cm X 20 cm). Enriched animals were housed in a group of 12 rats in a large three-level cage (75 cm X 75cm X 180 cm). Enriched animals had access to wheels and toys that were cleaned and replaced bi-weekly. The rats reared in isolation gained more weight than did enriched animals. Further, the animals in the enriched condition maintained lower rates of weight gain and overall lower weights for up to 20 weeks of age (Hellemans, Benge, & Olmstead, 2004). The only measure of physical activity was open-field locomotion (in a chamber other than the housing environment), examined on four consecutive days, after approximately 15 weeks of environmental enrichment. Animals reared in enriched environments had significantly less horizontal and vertical activity in the open field than did the animals reared in isolation. It is not clear if the enriched animals weighed less because they ate less, engaged in more activity in the home cages than did the isolation animals, or had higher metabolisms. The only physical activity data (locomotion) indicate that the enriched animals were less active than the isolated

animals, which would lead to the conclusion that enriched housed animals should weigh more, not less. The investigators did not report food consumption during rearing and did not measure **home cage** activity.

Tomchesson (2004) examined body weight, feeding, and open field activity in male, adolescent, Sprague-Dawley rats, housed in enriched and non-enriched environments. Rats were placed in an enriched or non-enriched environment for 24 days. In the non-enriched housing condition, animals were single-housed in standard polycarbonate rat cages with no objects in the cages (40 cm x 20 cm x 20 cm). In the enriched condition, animals were housed in groups of three, in larger polycarbonate cages (46 cm x 36 cm x 20 cm). A variety of objects (durable dog and cat toys including colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation. All of the animals were allowed access to a standard rat chow during the experiment. The non-enriched reared rats gained more weight than did the enriched animals and this effect was significant after approximately two weeks of enrichment. By the end of the 4 weeks, compared with rats housed in non-enriched environments, rats housed in enriched environments weighed less (6% on average) (Tomchesson, 2004). Results of the Open Field trials suggested that the animals in the enriched housing moved less than animals in non-enriched housing, which was consistent with previous open field data. This experiment did not examine **home cage** activity, it did not examine female subjects, and it did not examine various types of foods.

Together, these three experiments suggest that: (1) environmental enrichment can decrease body weight gains when bland foods are available but may have limited effect when cafeteria diets are available, and (2) open field physical activity does not appear to play a role in the effects of environmental enrichment on body weight. From these experiments, it is not clear (1) what role total activity, including activity in the home cage, may plan in controlling body weight, or (2) whether there are sex differences in environmental enrichment effects on body weight.

Potential Mechanisms of Action

There are several potential mechanisms for environmental enrichment to alter body weight. These mechanisms are directly related to the factors that can result in weight changes: genetics, metabolism, physical activity, and feeding. There is strong evidence for a genetic component to human obesity (e.g., the familial clustering and the high concordance of body composition in monozygotic twins). However, the role of genetic factors in human obesity is complex, determined by the interaction of several genes (polygenic) that work in combination with each other as well as with environmental factors (e.g., nutrients, physical activity) (Froguel & Boutin, 2001). There is no reported research examining environmental enrichment and genetics to suggest that environmental enrichment can modify body weight through genetic mechanisms in humans or animals. Genetic changes as a result of environmental manipulations typically have an extended time course that makes direct experimental examination unfeasible for this project. As such, detailed hypotheses regarding any potential

mechanisms of action for environmental enrichment to alter body weight through genetic variation is not reasonable to present at this time.

In contrast, there is evidence suggesting that environmental enrichment can affect metabolism. Metabolism is a combination of physical and chemical processes in which the body cells synthesize protoplasm for growth and repair along with complex substances that are broken down into simpler compounds that produce energy essential for the functioning of body cells (Hirsch, Fairchild, & Rosenbaum, 1995). Three factors determine metabolic rate: (1) basal metabolic rate (BMR), the normal rate at which the body uses energy without engaging in activity, (2) the total amount of calories the body uses daily, (3) the rate energy is burned during exercise and during the food digestion (Hirsch, Fairchild, & Rosenbaum, 1995). Factors that affect metabolism include body size, age, growth, gender, amount of lean muscle tissue, amount of body fat, hormonal and nervous system controls, and amount of physical activity (Baghurst et al., 1992).

One way to increase physical activity and potentially increase metabolism may be by providing additional room for movement. However, it is not clear that animals housed in environmental enrichment have higher levels of physical activity compared to animals housed in non-enriched environments. In fact, the limited available data (i.e., in novel open field environments) suggest that animals housed in enrichment are less active than animals housed in non-enriched environments. It is not clear if the animals take advantage of the opportunity for additional social and physical stimuli. An increase in physical activity would

result in energy expenditure. When expenditure exceeds intake, weight is decreased. If exposure to environmental enrichment results in increased activity and energy expenditure, then it would be logical that weight would decrease. The relationship between total physical activity and environmental enrichment is not known because it has not been previously examined.

Another potential mechanism for enrichment's effect on body weight is by directly influencing energy intake or feeding. The regulation of body weight is a homeostatic system involving circulating inhibitory signals that act on the brain to reduce or increase hunger which, in turn, increase or decrease appetite (Connan & Stanley, 2003). These inhibitory signals are generated in response to individual meals and can depend on the macronutrient content of the energy consumed. For example, Saris (2003) reported that there is an overwhelming amount of evidence that the ratio of fat to carbohydrate in the diet is the primary factor in the macronutrient composition of the diet that causes over-consumption and that leads to weight gain. In animals, sweet and salty foods are consumed in greater quantities than standard rat chow (Sclafani & Springer, 1976). The availability of different foods within the environment is a potential factor in body weight changes. It has been reported that animals have a high preference for activity and prefer exercise to feeding (Premack & Premack, 1963; Boakes & Dwyer, 1997). Environmental enrichment provides physical and social interactions, which also may alter food consumption. Because environmental enrichment affects feeding, the extent to which environmental enrichment affects consumption of foods, particularly more desirable foods, is important to examine.

Changes within the brain are also a potential mechanism for the effects of environmental enrichment on energy intake. The increased size of the frontal cortex, a consequence of environmental enrichment, may improve processing of the peripheral signals to the ARC and PVN within the hypothalamus, the center for energy consumption in the brain. For example, chemical signals from peptides (such as leptin or insulin) may be more quickly processed because the increased neurocircuitry (i.e., increased dendritic branching and synaptic formations) provides more receptors for such signals. It is also possible that endogenous hormones or neurotransmitters that affect feeding and appetite are altered as a result of environmental enrichment. For example, dopamine release was reportedly increased as a result of food deprivation (Berridge & Robinson, 2003). In a recent investigation in our laboratory, there was an increase in dopamine D2 receptors found in the brains of animals housed in environmental enrichment compared to animals housed in non-enriched conditions (Elliott, Grunberg, & Thanos, personal communication, 2005). Animals in enriched environments may alter energy intake as a result of changes in dopamine availability or other neurotransmitters.

Limitations in the Literature

The existing literature on rat models of environmental enrichment is extensive. However, few published experiments have examined environmental enrichment, body weight, food consumption, and physical activity. The few studies that have reported findings regarding these variables have three major limitations. First, no experiment examining the effects of enrichment on body

weight has directly compared male and female subjects. Males and females differ in their biology and react differently to environmental conditions (e.g., Cortright et al., 1997; Kolb et al., 1998; Curtis et al., 2004). For example, female rats housed in enriched environment have been reported to be more active than male rats housed in enriched environments in an open field arena (Elliot, 2004). There may be additional sex differences in the effects of environmental conditions on physical activity and body weight. The extent to which environmental factors may contribute to these behavioral differences has not been extensively examined, but may have important implications. Differences in feeding behaviors in response to environmental influences early in life may provide valuable information towards our understanding the current trend of childhood obesity in America.

Second, studies examining the effects of enrichment have not examined home cage activity of the animals. Physical activity is an important factor in whether or not an animal gains weight and the rate at which weight is gained. In studies of environmental enrichment, physical activity is typically measured only in response to being placed in a novel environment. The data from open-field locomotion suggest that environmentally enriched animals are less active and, therefore, should weigh more. This fact apparently opposes the effect of environmental enrichment to decrease body weight. One possible explanation is that enriched animals engage in more physical activity in their home cages. In contrast, it is possible that similar to their behavior in a novel environment, enriched animals habituate to their home cages, and that their activity in their

home cages decreases over time which would suggest a different cause for the decreased body weight (e.g., genetics, metabolism, feeding). Therefore, home cage activity in response to environmental enrichment needs to be systematically investigated.

A third limitation of environmental research on body weight is that the feeding behavior of adolescent animals reared in environmental enrichment has not been reported. The only investigation that reported the effects of environmental enrichment on food consumption used adult subjects (Sclafani & Springer, 1976). Sclafani and Springer (1976) reported that environmental enrichment did not offset overfeeding when adult rats were given access to a variety of foods. However, adolescent rats are particularly sensitive to the effects of environmental enrichment (Rosenzweig & Bennett, 1996; Kolb et. al., 1998) and feeding experiences of young rats has long lasting effects on food consumption as they age (Sefcikova & Mozes, 2002). Also, identifying whether different amounts of stimuli available in the environment interact with feeding behavior, activity, or gender may help to explain gender differences in body weight and may aid in the development of educational programs to treat overweight individuals. This doctoral research project was designed to address these limitations.

SECTION II - RESEARCH EXPERIMENTS

Overview

Three separate experiments were designed to examine the effects of environmental enrichment on feeding, activity, and body weight, in adolescent

male and female rats. Previous experiments reporting effects of enrichment on body weight had not: (1) examined home cage activity, (2) compared different enriched environments, and (3) compared the effects of different diets in male and female adolescent rats. This section begins with a description and rationale for each independent and dependent variable, a description of the experimental design and sample size, and an explanation of the data analytic strategy. Then, a description of the methods, results, and a discussion of the findings from each individual experiment are presented. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub, 82-23, rev. 1985).

Rationale for Independent Variables

Environmental Enrichment (EE)

The environment in which an animal lives has important and long-lasting biological and behavioral effects. In animal research, housing is a key factor that is often ignored because it is not a variable of interest to the investigator. In fact, a review of 339 peer-reviewed papers that reported animal investigations revealed that 43% of the papers did not mention cage size (Steyermark & Mueller, 2001). In addition to the size of the cage, the stimuli available in the cage also are important.

In their “cafeteria diet” study, Sclafani and Springer (1976) reported that environmental enrichment did not attenuate the rate of weight gain in adult female Sprague-Dawley rats when given access to a variety of foods. The

environmentally enriched condition, called a “complex environment,” housed eight littermates together in a large, three-level, wire mesh cage (45 cm X 47.5 cm X 67.5 cm) that included wooden, metal, and plastic objects in the upper two levels. An even larger environment reported in the literature is a series of three large interconnected wire mesh cages (72 cm X 70 cm X 46 cm each) (Soffie, Hahn, Tero, & Eclancher, 1999). These paradigms provide opportunities for social interaction and physical stimulation consistent with the concept of environmental enrichment (Rosenzweig & Bennett, 1996; Woodcock & Richardson, 2000). Enriched environments differ from isolated environments in the number of animals per cage and the number of objects per cage (Rosenzweig & Bennett, 1996; Kolb, et al., 1998; Van Praag, et a., 1999; Varty et al., 2000; Schrijver et al., 2002).

Three different forms of housing were used in the current research project: (Non-Enriched “NON”, Enriched “ENR”, and Super-Enriched “SUP”). The NON housing condition refers to housing animals singly in standard polycarbonate rat cages (40 cm x 20 cm x 20 cm) with no additional objects. The ENR housing condition refers to housing groups of three animals together in larger polycarbonate cages (46 cm x 36 cm x 20 cm) providing opportunities for social interaction. In addition, a variety of objects (durable toys including colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation. This particular enriched environment has been effective in previous experiments in our laboratory assessing environmental effects on performance and activity (Elliott, 2004; Tomchesson, 2004). The SUP condition

refers to a larger group housing where 12 animals are housed in a three-level, galvanized steel cage (76 cm x 61 cm x 137 cm) providing more opportunities for physical and social interaction than the ENR condition. A variety of objects (durable toys including colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation. This type of environment has been effective in experiments evaluating enrichment, feeding, body weight, and drug actions (Sclafani & Springer, 1976; Bowling, Rowlett, & Bardo, 1993; Hellemens et al., 2004). The two types of environmental enrichment were included in this project to determine if the amount of available physical and social stimulation differentially affects body weight in rats. It is possible that Sclafani and Springer (1976) did not provide enough space to make a difference on body weight with the cafeteria diet. Alternatively, enriched environments may not offset the effects of cafeteria diets on body weight.

Foods

The quality and quantity of available foods are important factors influencing energy intake, body adiposity, and body weight (Sclafani & Springer, 1976; Brown & Grunberg, 1995; Sclafani, 2001; Sefcikova & Mozes, 2002). High fat diets promote greater caloric intake and weight gain than lower fat diets based on epidemiological studies, experimental manipulation of dietary fat content in human participants, and animal models of diet-induced overeating (Warwick, Synowski, Rice, & Smart, 2003). By offering diets high in fat and/or sugar, overeating and obesity can be produced in rats (Sclafani & Springer, 1976).

Presumably, rats prefer diets high in fat and sugar because carbohydrates and fat taste better than bland or standard rat chow. Palatability and food taste have been reported as significant factors on total food consumption and weight gain in rats (Sclafani, 2004). However, other factors such as macronutrient content (e.g., protein, type of fat) (e.g., Levine, Kotz, & Gosnell 2003), age of specific nutritional experiences (e.g., Carughi, Carpenter, & Diamond, 1989; Sefcikova & Mozes, 2002), and housing (e.g., Brown & Grunberg, 1995; Lopek & Eikelboom, 2000; O'Conner & Eikelboom, 2000) also influence feeding behaviors.

Sclafani and Springer (1976) first reported that "sugar rich" and "tasty fat" foods marketed for human consumption produced pronounced weight gains in rats. This "cafeteria diet" allowed continuous access to foods that could be found in virtually any supermarket such as: sweetened condensed milk, chocolate chip and cream filled cookies, salami, cheese, bananas, marshmallows, milk chocolate, and peanut butter (Sclafani & Springer, 1976).   In fact, adult female rats given constant access to these foods gained over three times as much weight as did standard chow fed controls. Sweet and fatty foods have been reported to elicit approach behavior and reinforce operant responding (Sclafani, 2004; Berridge & Robinson, 2003). Therefore, human supermarket foods that are high in fat and sugar have robust effects on two key variables in this project: food consumption and body weight.

Environmental Enrichment and Foods

Few studies have reported the effects of environmental enrichment on feeding. Sclafani and Springer (1976) reported that environmental enrichment did not attenuate the weight gain of adult female, Sprague-Dawley rats when given access to a variety of foods commonly eaten by humans. Sclafani and Springer called this diet the “cafeteria diet.” Regardless of their housing condition (Complex [i.e., Enriched] or isolation [Non-Enriched]), animals fed the “cafeteria diet” ate more than did the standard chow fed rats and gained more weight. Although environmental enrichment did not attenuate the body weight gain when the animals had access to a variety of tasty foods, the animals in the complex environment that were given access to standard chow ate less than the animals in the isolated housing that had access to the standard chow. It is noteworthy that this experiment used adult rats, and the effects of environmental enrichment are typically investigated using adolescent animals because young animals are particularly sensitive to environmental enrichment (Rosenzweig & Bennett, 1996; Kolb et al., 1998). The present research included male and female adolescent rats to determine if environmental enrichment affects body weight of these subjects with access to various foods.

Additional evidence supports the effects of environmental enrichment to decrease food consumption. In an experiment examining the behavioral effects of environmental enrichment, 21-day-old male Sprague-Dawley rats were fed standard chow during a 4-week enrichment period. Animals in the environmental enrichment condition ate less than did animals in the non-enrichment condition

(Tomchesson, 2004). In this experiment, food consumption was used to monitor animal health and was not the primary focus of the research project.

Studies that did not directly examine environmental enrichment but manipulated components of an animal's environment have reported feeding changes. For example, paired housing (Lopak & Eikelboom, 2000), the introduction of a running wheel (Lattanzio & Eikelboom, 2003), alternating housing conditions (O'Conner & Eikelboom, 2000), and the use of smaller cages (Steyermark & Mueller, 2002) resulted in pronounced feeding suppression. It is clear from the literature that environmental manipulations can significantly affect feeding. While enriched environments appear to decrease the body weight of animals, it is unclear if the reduction in body weight is the result of decreased feeding or energy intake, or the result of an increase in activity. The available open-field data suggest that activity is decreased in enriched animals; however, no studies of environmental enrichment have examined feeding and home cage activity. The present experiments include measures of food consumption and activity in the home cages in addition to activity in open-field chambers.

Sex

Males and females differ in their biology and they react differently to environmental conditions (e.g., Cortright, Chandler, Lemon, Lemon, & DiCarlo, 1997; Kolb, Forgie, & Gibb et al., 1998; Curtis et al., 2004). In humans, adult males have been reported to experience significant weight loss with exercise despite only mild energy deficits (Anderson et al., 1991). Conversely, females who regularly exercise typically maintain a stable weight despite more substantial

energy deficits (Mulligan & Butterfield, 1990). In animals, female rats have been reported to have a greater preference for salt (NaCl) and sweet solutions compared to male rats (Flynn, Schulkin, & Havens, 1993). Curtis and colleagues (2004) reported that female rats are less sensitive to higher concentrations of sodium and less sensitive to lower concentrations of sucrose solutions compared to male rats, suggesting gender differences in taste preferences. Gender differences also have been reported in activity. Female rats subjected to exercise training (swimming, treadmill) gain weight at approximately the same rate, as do sedentary controls (Applegate, 1982). Conversely, males subjected to regular exercise typically lose weight (Oscai & Holloszy, 1969). Additionally, male rats allowed daily access to a running wheel reduced body mass and body fat compared to female rats allowed the same access to a running wheel.

Sex differences in feeding behavior have been reported in the animal research literature with some mixed results. Grunberg, Popp, and Winders (1988) investigated the effects of nicotine on body weight, consumption of potato chips, cookies, and standard chow in rats. Prior to the experimental manipulation of nicotine, adult female Sprague-Dawley rats ate less bland food than did adult male, Sprague-Dawley rats (4.45 g vs. 10.66 g, respectively) (Grunberg, Popp, & Winders, 1988). Further, there were no significant differences in the types of foods consumed by males and females. Males and females ate similar amounts of cookies (11.42 g vs. 10.44 g) and potato chips (2.32 g vs. 2.14 g) (Grunberg, Popp, & Winders, 1988). Additionally, adult female rats fed high-fat and high-carbohydrate diets ate more high-fat foods and became fatter than adult male

rats (Sclafani & Gorman, 1977). Given the recent trend of excessive body weight currently being reported in adolescents, it is important to examine consumption of various types of foods in adolescents. It also is important to determine whether environmental enrichment alters these behaviors.

Enrichment Effects and Sex Differences

The effects of enriched environments have been investigated primarily using male subjects. A few studies have compared the performance of males and females raised in enriched and non-enriched environments. Male and female rats raised in enriched environments exhibit enhanced performance on a reference memory task compared to rats raised in non-enriched environments (Einon, 1980). Male and female rats raised in enriched environments also made fewer memory tasks errors than did rats raised in non-enriched environments (Seymore, Dou, & Juraska, 1996). Examining spatial memory performance using only females, Daniel, Roberts, and Dohanich (1999) have obtained similar results.

The effects of social environment on locomotion, feeding, acoustic startle, and pre-pulse inhibition (PPI) in male and female Sprague-Dawley rats suggest that females are more sensitive than are males to the behavior-altering effects of group housing (i.e., social enrichment) (Brown & Grunberg, 1995; Faraday, Rahman, Scheufele, & Grunberg, 1998; Faraday, Scheufele, Rahman, & Grunberg, 1999). Results of a recent study conducted in our laboratory suggest that males and females differ in their sensitivity to physical and social aspects of the environment. Elliott (2004) reported that males and females responded

differently to the effects of social and physical enrichment on a variety of tasks of information processing (i.e., acoustic startle response and pre pulse inhibition), simple working memory (i.e., passive avoidance), and complex spatial learning and memory (i.e., Morris water maze). Social enrichment enhanced performance of males and females on a simple information-processing task (i.e., locomotor habituation). Physical enrichment enhanced performance on the same measure for males, but not for females. Social enrichment improved performance on a complex spatial memory (i.e., Morris water maze) for males, but not for females. Physical enrichment improved sensory gating (i.e., % PPI) for females, but not for males. The present research project, therefore, included male and female subjects.

Rationale for Dependent Variables

Body weight (BW)

Body weight (BW) gain is the net result of daily energy intake and energy expenditure. BW increases when the amount of daily energy intake exceeds the amount of daily energy expended (Boon, Visser, Daan, 1997; Warwick, Synowsky, & Bell, 2002). In animals, BW is a basic biologic measure that can be used to index health, monitor aging, and evaluate growth (e.g., Sclafani and Springer, 1976; Brown & Grunberg, 1995; Faraday & Grunberg, 2000). Within the rodent literature, experimental subjects are typically described using two criteria: (1) the age of the animal reported as the number of days since birth (e.g., 21 days old), and (2) the animal’s body weight in grams (e.g., 300 grams).

BW is a widely used measure in studies of: social isolation (Hall, Humby, Wilkinson, & Robbins, 1997), social crowding (Brown & Grunberg, 1995), physical and social stimulation (Sclafani & Springer, 1976; O'Conner & Eikelboom, 2002), immobilization stress (Faraday, 2002), and various pharmacologic agents (Grunberg et al., 1985; Bowling et al., 1994). Of particular interest to this project, studies examining feeding behaviors that manipulate food availability or food/nutrient choice routinely use BW as a dependent variable (e.g., Sclafani & Springer, 1976; Hall, Humby, Wilkinson, & Robbins, 1996; Boakes & Dwyer, 1997; Lopek & Eikelboom 2000; Sefcikova & Mozes, 2002; Lattanzio & Eikelboom, 2003).

In addition, BW can be used to monitor animal health. For example, "crowding" animals can provide stress (Bowen & Grunberg, 1995) or competition for food that prevents animals from receiving necessary nutrients. A continuous lack of nutrients can result in poor health and retarded growth. Insufficient nutrition that occurred in young rats during their first three weeks of life resulted in decreased body weight and decreased body growth when compared to normally fed controls (Bartness et al., 1987). Conversely, excess body weight can indicate poor health. Excessive weight gain can be induced in rats by offering them foods that are high in fat and sugar through foods usually used for human consumption such as cream filled cookies, marshmallows, milk chocolate, potato chips, and peanut butter (Sclafani & Springer, 1976; Grunberg, Bowen, Maycock, & Nespor, 1985; Sclafani 2004).

Excessive weight gain increases risk for diseases such as coronary heart disease, diabetes, hypertension, respiratory problems, and some types of cancer (Flegal et al., 2002). BW is a simple, non-invasive measure that is sensitive to environmental manipulations. Additionally, BW is a face-valid measure used with humans and animals that can be measured repeatedly in the same animals and analyzed using the mixed experimental design (Brown & Grunberg, 1995; O'Conner & Eikelboom, 1999; Faraday, 2002).

Body mass index (BMI)

BMI is a measure expressing the relationship (or ratio) of weight-to-height commonly used to classify a human's weight as healthy or unhealthy. BMI is calculated by using the following formulas:

$$\text{BMI} = \underset{\text{(in Kilograms)}}{\text{Body Weight}} \div \underset{\text{(in meters)}}{\text{Height}^2} \quad \text{OR} \quad \text{BMI} = 705 \text{ X } (\underset{\text{(in pounds)}}{\text{Body Weight}} \div \underset{\text{(in inches)}}{\text{Height}^2})$$

The "height" of the rat was measured from the tip of the nose to the end of the rump defined as the beginning of the tail. BMI is more highly correlated with body fat than any other indicator of height and weight (NRC, 1989). Further, BMI is a reliable and valid measure of adiposity in adults (Garrow & Webster 1985) and children (Zimmermann, Gubeli, Puntener, & Molinari, 2004).

In rodents, few studies have examined BMI. Increases in BMI are correlated with increasing levels of leptin in rats (Maffei et al., 1995; Engelbregt et al., 2001**)**. The hormone leptin is the central mediator in a negative feedback loop regulating energy homeostasis (Engelbregt et al., 2001**)**. Leptin

administration leads to reduced food intake, increased energy expenditure, and weight loss **(**Cohen & Friedman, 2004). Additionally, weight loss as a result of food restriction has been associated with a decrease in plasma leptin (Maffei et al., 1995). Engelbregt et al. (2001) reported that BMI was correlated with body mass and percentage of fat in 24 day old female but not male Wistar rats. BMI is easily obtainable and has been correlated with the percentage of body fat in rats (Maffei et al., 1995**)**. This research project used the formula presented by Engelbregt and colleagues (2001) to calculate BMI:

$$\boxed{\mathrm{BMI} = \underset{\text{(in grams)}}{\text{Body Weight}} \div \underset{\text{(in cm)}}{\text{Height}}^2}$$

Lee Index (LI)

Similar to BMI, the LI is a ratio of weight-to-height used to report adiposity in rats. It is a non-invasive and simple measure that is used in rodent research on obesity and feeding. Using the same measurements taken to calculate the animal's BMI, LI is the cubic root of body weight in grams divided by the naso-anal length in millimeters times 10^4. The naso-anal length of the rat is measured from the tip of the nose to the end of the rump, defined as the beginning of the tail. LI is highly correlated with body fat and has proven to be a reliable and valid measure of adiposity in rats (Lee, 1929; Bernardis, Luboshitsky, Bellinger, & McEwen, 1982; Emsberger, P., Koletsky, P.J., Baskin, J. S., & Collins, L.A., 1996).

Several studies have reported LI as a reliable measure of adiposity. Straub (2003) reported that adult Wistar rats that were fed a normal diet and

administered drugs to reduce weight over a four-week period had significantly lower LI scores compared the animals that did not receive the weight-reducing drugs. Bernardis and colleagues (1982) reported that Lee Index scores for obese male Sprague-Dawley rats (that received bilateral electrolytic lesions in the ventromedial hypothalamic nuclei [VMNL rats] to induce obesity) were greater than were LI scores of control rats (that received a sham-operation). In a population of normal and obese mice, Rogers and Webb (1980) reported that the Lee Index correlated significantly with body fat and several other measures used to indicate obesity in mice (body density, body water, and proportional weight of the gonadal fat pad). This research project used the formula presented by Lee (1929) to calculate the LI scores:

$$\boxed{\text{LI} = [(\text{g body wt})^{1/3} / (\text{mm body length})] \times \text{l0}^{4}}$$

Food consumption (FC)

Similar to body weight (BW), food consumption (FC) can affect health. Extensive experimental data indicate that feeding behaviors can be profoundly affected by environmental manipulation. For example, stressors such as the tail pinch (Faraday, 2002) and repeated cold stress (Schultz et al., 1999) increase food intake, whereas restraint (Zylan & Brown, 1996; Faraday, 2002) and crowding (Brown & Grunberg, 1995) reportedly decrease feeding.

The role of housing in feeding behaviors is not as clear. Perez et al. (1997) reported that individually housed rats ate more than did rats housed in groups. In contrast, moving rats from individual to paired housing has been

reported to decrease food intake for several days and feeding was not altered when the rats were placed from their paired housing back into individual housing (O'Conner & Eikelboom, 2000). Placing rats in isolation at the weaning period increased food consumption (Fiala, Snow, & Greenough, 1977), but placing rats in isolation after weaning has been reported to decrease feeding (Yamada et al., 2000). The feeding behavior of rats appears to be particularly sensitive to early environmental manipulation. When mothers were insufficiently nourished, consequent malnourishment of the rat pups led to changes in feeding when the pups were adults. Specifically, males that were underfed during lactation feeding ate more standard rat chow during adulthood compared to the well-fed controls (Smart & Dobbing, 1977). Undernourished female pups were smaller and weighed less in adulthood, but did not significantly over-eat on a diet of standard rat chow (Sefcikova & Mozes, 2002).

Although the directionality of environmentally-driven feeding changes may not be clear, the fact that feeding changes are sensitive to environmental manipulations is important. If a clearly established feeding pattern is altered by a given manipulation, then it is clear that the manipulation has produced a change in behavior. This change in feeding can have health consequences, regardless of whether the resultant change is an increase or decrease in feeding. These reported differential responses in feeding parallel human feeding. For example, in the DSM IV T/R (2001), one criterion for depression is a change in feeding and body weight -- Directionality is not an important distinction. Further, stress and anxiety can result in increased or decreased appetite (Zylan & Brown, 1996;

Brown & Grunberg, 1995; Faraday, 2002). Again, the difference from baseline consumption is the key feature of the criterion (DSM IV T/R, 2001).

FC is a simple, non-invasive measure that is sensitive to environmental manipulations. Additionally, similar to BW, FC is a face-valid measure used with humans and animals (Brown & Grunberg, 1995; O'Conner & Eikelboom, 1999; Faraday, 2002) that can be measured repeatedly in the same animals and analyzed using the mixed experimental design of the present experiments.

Physical Activity (PA)

Home cage activity (HCA)

Home cage activity refers to the animal's behavior in its primary living quarters. Previous researchers have reported a seemingly paradoxical finding that rats reared in environmental enrichment compared to rats reared in non-enriched environments appear to have less activity (at least in novel environments) (e.g., Van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998) but weigh less (O'Conner & Eikelboom, 2000; Hellemans, Benge, & Olmstead, 2004). It is noteworthy to mention that these "enriched" rats were healthy animals because rats that were ill would be expected to be less active, eat less, and, therefore, weigh less. Consequently, it was important to examine if animals reared in the enriched and non-enriched environments differ in the amount of activity within their home cages. An extensive literature search of the PsychInfo® and National Library of Medicine (PubMed®) databases revealed no studies that have examined home cage activity in enriched environments.

Therefore, two observational methods were developed and used to assess home cage activity. The two methods are described in the methods of Experiment I.

Open field (OF)

Open-field locomotion describes an animal's behavior when it is placed in a non-home cage arena. Behaviors relevant to environmental enrichment include activity in the horizontal plane, vertical plane, and center time. Level of activity and frequency of rearing behaviors have been used to index an animal's habituation to a novel environment (Varty et al., 2000; Bowling et al., 1993; Van Waas & Soffie, 1996). Habituation, a simple form of learning, refers to the progressive reduction in responding to a novel stimulus when the stimulus is repeatedly presented to a subject (Varty et al., 2000). A decrease in overall activity or rearing behaviors suggests habituation or efficient processing of novel information. Conversely, an absence of behavioral changes over time reflects decreased information processing of relevant environmental stimuli (Varty et al., 2000; Bowling et al., 1993; Van Waas & Soffie, 1996).

Relevant to this project, animals housed in enriched environments reduce locomotor activity, reduce exploration over time, and have more restricted movements in open field testing when compared to non-enriched animals (van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001; Varty et al., 2000). These findings suggest that rats housed in enrichment assimilate information from their environment and adapt more effectively to novel environments than do rats raised in non-enriched environments. This decrease in activity, however, is not consistent with the

research reported that environmental enrichment decreases the rate of weight gain in rats (O’Conner & Eikelboom, 2000; Hellemans, Benge, & Olmstead, 2004; Tomchesson, 2004). Based on energy expenditure alone, if activity decreased, then the weight of the animals should increase. Home cage activity has not been carefully examined and compared to open-field activity within the context of environmental enrichment. This research project examined the effects of environmental enrichment on activity in both the novel open-field environment and home cage.

Experimental Design and Determination of Sample Size

All three experiments were conducted using a 7-week, repeated-measures, mixed (i.e., within-subject and between-subjects) factorial, A-B-A design with 12 subjects per cell. Experiment I and Experiment II examined the effects of environmental enrichment on feeding, weight gain, and activity in male and female adolescent Sprague-Dawley rats, respectively. Experiment III examined male and female subjects together to attempt to replicate the findings from the first two experiments and to allow a direct comparison of sex. Animals were housed in one of three types of housing, representing three different levels of environmental enrichment: Non-Enriched (NON), Enriched (ENR), and Super-Enriched (SUP). All subjects were given access to different types of foods during the different phases of the experiment (A = Standard Food, B = Standard Food plus Salty plus Sweet Junk Foods). During Phase A, all subjects had access to standard rat chow. During Phase B, all subjects had access to standard rat chow a sweet food (Oreo™ cookies), and a salty food (Lay’s™ plain potato chips).

Three behavioral measures were used: open-field locomotion, home cage activity, and food consumption. In addition body weight was measured and a body mass index score and a Lee Index score were calculated for each animal at the end of each study.

The sample sizes were determined based on previous reports using similar dependent measures and responses to environmental enrichment. Similar studies in the research literature, which reported statistically significant effects, had cell sizes of 7 – 12 animals for enrichment (e.g., Van Praag et al., 1999; Elliott & Grunberg, 2005; Tomchesson, 2004). Mering Kaliste-Korhonen and Nevalainen (2000) determined that 5 - 10 animals were needed to find statistically significant effects for enrichment on various biological measures including body weight and fat/adipose tissue. The exact experimental design and sample size of Experiment III was determined after completing Experiment II.

A sample size of 12 animals was selected to optimize statistical power across a range of dependent measures that vary in effect size in response to environmental enrichment and weight and was determined based on studies in the existing literature. Sample sizes were confirmed using the procedures of Keppel (1991) and Cohen (1988). Estimates of effect size in the population were determined to provide 0.80 power by calculating an estimated omega squared (ϖ^2) according to the formula:

$$\varpi^2_A = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_{S/A}}$$

where ϖ^2_A refers to the estimated population treatment effects and $\sigma^2_{S/A}$ refers to the estimated population error variance (Keppel et al., 1992, p. 180). The omega

squared statistic provides a measure of effect size that is relatively independent of sample size and is expressed as a proportion of the total variability (σ^2_A + $\sigma^2_{S/A}$) that is associated with the treatment or manipulation (σ^2_A).

Data Analytic Strategy

The goal of this project was to examine if environmental conditions affect activity, feeding, body weight, body fat, and the extent to which any effects may vary as a function of sex. For all animals, food consumption (FC), BMI, and LI were analyzed with separate analyses of variance (ANOVAs) with Enrichment (housing condition) as the between-subjects factors. Body weight (BW) was analyzed with repeated-measures analyses of co-variance (ANCOVA) with Enrichment as the between-subjects factor and Time as the within-subject factor. Baseline body weight was used as a covariate to compensate for the variance of body weights within each experimental group throughout the various experiments. Home cage activity (HCA) was analyzed using the Kruskal-Wallis chi-square because HCA data were interval data with more than two independent experimental groups. For the open-field (OF) measures, horizontal and vertical activity was first analyzed with a multivariate analysis of variance (MANOVA) to determine if there was an overall difference between groups across the two related OF measures. If the MANOVA results revealed significant differences on the OF measures, then separate univariate analyses of variance (ANOVA) for each OF variable were performed. During the analyses, Tukey HSD tests or, when a covariate was used, a pairwise post-hoc comparison using a Bonferroni correction for multiple comparisons was used to compare housing conditions if

there was a significant main effect for housing type. The computer-based statistical analyses automatically adjusted the alpha levels following the Bonferroni correction procedures to maintain a true alpha of 0.05. For Experiment III, a repeated-measures analysis of variance (ANOVA) was used with Sex and Enrichment (housing condition) as the between-subjects factors and Time as the within-subject factor.

All tests were two-tailed with alpha = 0.05. Several strategies were employed to minimize the probability of Type 1 error. First, the experiment was designed with enough subjects to provide adequate power (i.e., 0.80). When the sample size is large enough to support adequate power, the likelihood of Type I errors is minimized. Second, global analyses incorporating all factors (Housing Condition and Sex) were used to guide internal analyses. Sub-group analyses followed only if overall analyses revealed significant main effects or interactions. This strict Fisherian strategy is consistent with recommendations of Keppel (1991) and Cohen (1988), and reduces the number of statistical tests performed. Finally, the error term (the within-subject variance that constitutes the denominator of the F ratio) specific to the comparison being made was used rather than the error term for all subjects. This technique controls Type I error because as the denominator degrees of freedom decrease, the F value necessary to achieve significance for a given comparison increases. The following section presents the experimental results for each dependant variable and provides an individual discussion of the results for each of the three experiments in this project. *Note:* The following abbreviations are used for the

three housing conditions: “NON” = Non-Enriched; “ENR” = Enriched; “SUP” = Super-Enriched housing.

Experiment I

Experiment I examined the effects of environmental enrichment on activity, feeding, and body weight in male, adolescent, Sprague-Dawley rats. The experiment was a 7-week investigation that used a repeated-measures, mixed, A-B-A design. Subjects were housed in one of three environmental conditions (NON, ENR, and SUP) for the duration of the experiment and all subjects were given access to different types of foods during the different phases of the experiment (A = Standard Food, B = Standard Food plus Salty plus Sweet Junk Foods). The goals of Experiment I were to examine the effects of environmental enrichment on: (1) body weight when different foods are available, (2) food consumption (variety of foods), (3) physical activity (open field arena and home cage).

Hypotheses: Experiment I

Hypothesis 1 – EE, BW, BMI, and LI

A) Environmental enrichment will decrease body weight gains, Lee index, and Body Mass index such that: Non-Enriched > Enriched > Super-Enriched.

Rationale. Environmental enrichment has been reported to decrease the rate of weight gain in rats (O’Conner & Eikelboom, 2000; Hellemans, Benge, Olmstead, 2004; Tomchesson, 2004).

Hypothesis 2 – EE and FC

A) Environmental enrichment will decrease the amount of bland foods consumed compared to animals reared in Non-Enrichment such that: Non-Enriched > Enriched > Super-Enriched.

Rationale. Rats housed in environmental enrichment eat less bland food (Sclafani & Springer, 1976; Tomchesson, 2004).

B) Animals raised in enriched and non-enriched housing conditions will eat similar amounts of cafeteria foods such that: Non-Enriched = Enriched = Super-Enriched.

Rationale. Rats housed in environmental enrichment did not eat less cafeteria food than singly housed controls (Sclafani & Springer, 1976).

Hypothesis 3 – EE and PA

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Non-Enriched > Enriched > Super-Enriched.

Rationale. Enriched animals exhibit reduced locomotor activity and reduced exploration over time compared to non-enriched animals (Van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001; Varty et al., 2000).

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Non-Enriched < Enriched < Super-Enriched.

Rationale. Animals exposed to longer activity periods have higher daily energy expenditure (Boon, Visser, Daan, 1997) and animals that are allowed access to exercise (i.e., a running wheel) choose exercise over feeding (Premack & Premack, 1963; Boakes & Dwyer, 1997; Mueller, Herman, & Eikelboom, 1999; Lattanzio & Eikelboom, 2003). Therefore, the animals in the enriched housing conditions that have more opportunity for physical activity will be more active.

Methods: Experiment I

Subjects

Subjects were 36 adolescent male (21 days old at the beginning of the experiment) Sprague-Dawley rats (Charles River Laboratories). Adolescent animals were used to maximize the developmental impact of environmental environment and because of the investigator’s interest in child and adolescent development. Sprague-Dawley rats were used because they are the most commonly used strain of outbred albino rats. Twelve subjects were randomly assigned to each of the three experimental treatment conditions upon arrival.

Housing

All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at approximately 23^0 C and approximately 50% relative humidity on a 12-hour reversed light/dark cycle (lights off at 0530 hours). A reversed light cycle was maintained to ensure that all behavioral measures were made during the animals’ normal activity period. Animals were assigned to one of three housing conditions (Non-Enriched “NON”, Enriched

"ENR", and Super-Enriched "SUP"). In the NON condition, animals were single-housed in standard polycarbonate rat cages (40 cm x 20 cm x 20 cm) with no additional objects. In the ENR condition, animals were housed in groups of three in larger polycarbonate cages (46 cm x 36 cm x 20 cm), which provided opportunities for social interaction. In addition, a variety of objects (durable toys including colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation. Objects were removed every other day (or sooner if damaged) and replaced with new objects. In the SUP condition, 12 animals were housed together in a three-level, galvanized steel cage (76 cm x 61 cm x 137 cm), which provided more opportunities for physical activity and social interaction than the ENR condition. A variety of objects (durable toys including colored textured balls, rings, and bones) also were placed in the cage to provide physical and tactile stimulation. The objects used, changing schedule, and cage dimensions were based on methods described in previous studies (Gardner et al., 1975; Sclafani & Springer, 1976; Bowling et al., 1993; Varty et al., 2000; Elliott, 2004; Tomchesson, 2004). See Figures 1-3 in the Appendix for pictures of housing conditions.

Procedures

On experimental Day 1 (upon arrival to the laboratory), animals were randomly assigned to one of the three housing conditions for the experiment's duration. The experiment was conducted in three experimental phases using an A-B-A design. Each experimental phase corresponds to a change in the animal's

available food. All animals, regardless of housing condition, had access to the same foods.

The first two experimental phases each lasted two weeks, and the third phase lasted three weeks. The entire experiment lasted a total of 50 days. Table 1 in Section IV presents the experimental Timeline. During Phases A1 and A2 (Days 1-14 and 30-50, respectively), all animals had access to standard rat chow. During Phase B (Days 15-29), all subjects were allowed to eat standard rat chow and, in addition, had access to a sweet food (Oreo™ cookies) and a salty food (Lay's™ plain potato chips). The letters A and B identify the experimental phase, the numbers 1 and 2 differentiate between the two A phases, and the number of days spent in the phase designate the experimental days during each of the phases (e.g., A1-1 is the first day of the first phase where animals received standard chow). All animals were acclimated to the open-field (OF) activity chambers on Day 2 to minimize contamination of responses by any stressful effects of exposure to a novel situation (Faraday & Grunberg, 2000). Acclimation procedures do not affect later measurement of OF habituation. All behavioral measures were conducted between 0800 and 1300 hours (the dark/active cycle). This period of time was used to maximize behavioral performance and physical activity because rats are nocturnal and more active during the dark cycle.

Dependent Variables

Body Weight (BW). BW was measured on Day 1 and then three times during each two-week experimental phase (the 5th, 10th, and 14th day) and two

additional times for Phase A2 (the 18th and 21st day). Animals were removed from their cages and gently placed on an electronic scale. To ensure accurate weight measurements (i.e., reduce measurement error), the electronic scale automatically obtains multiple weight readings and provides an average of these readings. These procedures provided 12 body weight measures (four during Phase A1, three during Phase B, and five during Phase A2).

Body Mass Index (BMI). BMI was measured on the final day of the experiment. At the end of the experiment, body weight (BW measurement 12) and naso-anal length in millimeters were measured and documented. BMI was calculated using these values.

Lee Index (LI). LI was measured on the final day of the experiment. At the end of the experiment, body weight (BW measurement 12) and naso-anal length in millimeters were measured and documented. LI was calculated using these values.

Food Consumption (FC). Food weights were measured every other day providing a total of 21 Food Consumption measurements. Animals had continuous access to food. Standard, bland, food pellets were placed on the top of each cage for NON and ENR conditions and in four separate food containers placed inside the cage for the SUPER condition. The salty and sweet foods were provided in 4 inch metal food cups with lids and placed in the cages in alternating positions to avoid the development of place preferences or aversions. Food Consumption was determined by subtracting new food weights from previous food weights (e.g., subtracting Day A1-3 food weights from Day A1-1).

Whenever food was added to the containers, the new weight was recorded and this new weight was used in the next calculation. Six Food Consumption values were calculated for the animals during each two-week experimental phase (nine values calculated for Phase A2). To appropriately describe food consumption for this investigation, both the weight of food consumed (in grams) and caloric intake during each experimental phase were examined. The number of grams consumed was examined to provide a simple measure to compare the amount of food eaten. The number of calories consumed was examined to allow a direct comparison of energy intake (caloric density of foods can vary depending on weight and macronutrient content of the foods). Each variable was examined separately during each phase because the available foods differed during each phase (standard chow for Phases A1 & A2 and standard chow, salty, and sweet during Phase B).

Physical Activity (PA). Two different methods of behavioral observation were developed to examine activity in home cages. The first method (Individual Animal Activity) was designed to examine animal activity for each individual animal. The second method (Group Activity) was designed to examine an aggregate level of activity for each experimental housing group. Each method is described in detail. (See figures 4 and 4a for HCA Data Sheets.)

Home Cage Activity (HCA) Method 1 – Individual Animal Activity. Individual animal activity was observed three times during the first two-week experimental phase of Experiment I (the 2nd, 7th, and 14th day). Two independent observers quietly observed each animal in home cages. The room was dimly lit

with red light. Each observer watched each animal for 3 minutes and recorded horizontal and vertical locomotion on a 7-point Likert format scale. The order of rats observed was balanced across housing conditions and the time of observation was varied during the dark cycle for each day of observation. The level of effort expended during each activity period also was judged and rated on a 7-point Likert format scale. In addition, the type of physical activity that each animal was engaged in was recorded (e.g., with a physical object, combined social and physical interaction, or alone).

This technique was initially tested using a small group of five adult animals from a previous experiment in our laboratory. The two independent observers were trained until an inter-rater reliability of at least 80% was obtained for at least five consecutively observed animals. Individual activity was to be examined at least three times during each experimental A-B-A phase (the beginning, middle, and end). However, it became immediately evident that this measure was not providing meaningful data. In less than 10 minutes after the observers entered the housing room, all of the animals appeared to decrease their activity levels. Within 30 minutes after entering the room, typically all of the animals would stop moving and lay down. In addition, it was nearly impossible for the observers to keep track of all of the animals' individual activity in the larger cage using this Individual Animal Activity technique.

The observers also tried leaving the room for over an hour and then returning to observe activity again. When the observers returned, the animals were active again and the observers again attempted to record activity. This

time, the observers quickly recorded a rating of “overall” activity for the group of animals in each experimental housing condition. The rationale was for the observers to obtain a “snap shot” of physical activity as soon as they entered the room, prior to attempting to get individual animal activity ratings. As before, the animals’ physical activity ceased within 30 minutes after the observers entered the housing room. However, preliminary analyses examining the “group” activity measures suggested that group activity provided meaningful data with which to compare the three experimental housing groups. An additional attempt to measure individual animal activity was made and a group activity measure was recorded prior to individual observations. Once again, the group activity recordings provided meaningful data to compare the experimental groups. Therefore, a “group activity” scale was developed and used for the remainder of Experiment I.

Home Cage Activity (HCA) Method 2 – Group Activity. Group activity was observed a minimum of five times during experimental phases B and A2. The measure was developed during the first phase, so no group activity measures are available for Phase A1. Two independent observers quietly observed animals in home cages and provided a global rating for each experimental housing group. The room was dimly lit with red light. Each observer watched each animal for one minute and recorded the number of animals engaged in physical activity, and average horizontal, vertical, and center cage locomotion activity on a 7-point Likert format scale. An average level of effort expended during each activity period also was judged and rated on a 7-point Likert format

scale. In addition, the type of physical activity that each animal was engaged in was recorded (e.g., with a physical object, combined social and physical interaction, or alone). The order of rats observed was balanced across housing conditions and the time of observation was varied for each day of observation. The observations all were made during the 12-hour dark cycle, but the observation time within this cycle was varied for each observation day.

Group activity was observed at random times to sample activity. The activity was monitored during the animals' dark cycle/active period to maximize the potential to observe the animals engaging in physical activity. In addition, the observers alternated the order in which the groups were observed to reduce any biases and confounds associated with the amount of time the observers were in the room (as seen when the observers were recording individual activity). These procedures were used for the remainder of Experiment I.

Open-Field (OF). OF activity was measured at the beginning and end of each experimental phase (Days A1-4, A1-13, B1-3, B1-13, A2-3, and A2-20). Open-Field activity was measured using an Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM [16 TAO]; Omnitech Electronics, Columbus, OH). Animals were placed singly in a 40 x 40 x 30 cm clear Plexiglas arena and a Plexiglas lid with multiple 3.5 cm diameter holes was placed on top of the arena. The lid ensures that subjects have adequate ventilation but cannot escape during data collection. A photocell array measures horizontal activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2

cm above the arena floor. A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measures vertical activity. Data are transmitted to a computer via an Omnitech Model DCM-I-BBU analyzer. (See Figure 6 in Section IV for a picture of an OF arena.)

Once subjects are placed in the test arenas, the experimenter turns off the lights and leaves the room. The apparatus monitors animal activity continuously for a total testing period of 1 hour. The interfaced software generates 21 sub-variables, including horizontal and vertical activity (measures of activity in the horizontal plane and exploratory activity, respectively). Horizontal activity and vertical activity were analyzed as measures of general activity, habituation, and exploration.

Results: Experiment I

Body Weight (BW)

Body weight was measured 12 times during the experiment (four times during Phase A1, three times during Phase B, and five times during the Phase A2). An overall ANCOVA revealed a main effect for Time (F (10, 320) = 8.185, p < 0.05), main effect for housing (F (2, 32) = 3.474, p < 0.05), and a Time X Housing interaction (F (10, 320) = 4.601, p < 0.05). Post hoc comparisons revealed that animals housed in Non-Enriched cages weighed more than animals in Enriched cages that weighed more than animals in the Super-Enriched cages (NON>ENR>SUP). Post-hoc pairwise comparisons using a Bonferroni adjustment for multiple comparisons revealed that animals housed in Super-Enriched cages weighed significantly less than animals housed in Non-Enriched

cages, but the differences in the weights of animals housed in Non-Enriched vs. Enriched housing as well as Enriched vs. Super-Enriched were not significant (NON≥ENR>SUP). There were no significant differences in body length between the three groups (See Table 22). (See Table 2 and Figures 7a- 7c for Body Weight results.)

Body Mass Index (BMI)

BMI was computed based on body weight and length at the end of the experiment corresponding BW measurement 12. BMI values were significantly related to housing conditions (F (2, 33) = 6.682, $p < 0.05$). Tukey HSD post hoc analyses revealed that animals in the NON housing condition had significantly higher average BMI than the animals raised in the ENR condition, which had higher average BMI than, but was not significantly different from, the SUP condition (NON>ENR≥SUP). (See Table 3 and Figure 8.)

Lee Index (LI)

LI was computed based on body weight and length at the end of the experiment using measurement 12. Animals housed in enrichment had significantly lower LI values compared to animals housed in no enrichment (F (2, 33) = 4.919, $p < 0.05$). Tukey HSD post hoc analyses revealed that animals in the NON housing condition had significantly higher average LI values than the animals raised in the ENR condition, which had higher average LI values that were not significantly different from the SUP condition (NON>ENR≥SUP). (See Table 4 and Figure 9.)

Food Consumption (FC)

FC was measured 25 times during Experiment I (seven measurements for Phase A1 & B and 11 measurements for Phase A2). Average grams and calories consumed daily were examined separately for each experimental phase because the available foods differed for each phase (i.e., during Phases A1 & A2, only standard chow was available and during Phase B, standard chow, salty, and sweet foods were available). The results for grams and calories are presented separately for each phase of the experiment.

Phase A1 – Standard Chow Only Period: GRAMS. There was a significant main effect for housing (F (2, 33) = 3.835, p < 0.05). A Tukey HSD comparison revealed that the difference between animals housed in Super-Enriched conditions and animals housed in both Non-Enriched and Enriched conditions approached significance (0.056 and 0.057, respectively), but there was no difference between animals raised in Non-Enriched and Enriched conditions (NON$\geq$ENR>SUP). (See Table 5a and Figure 10a.)

Phase A1 – Standard Chow Only Period: CALORIES. There was a significant main effect for housing (F (2, 33) = 3.835, p < 0.05). A Tukey HSD comparison revealed that the difference between animals housed in Super-Enriched conditions and animals housed in both Non-Enriched and Enriched conditions approached significance (0.056 and 0.057, respectively), but there was no difference between animals raised in Non-Enriched and Enriched conditions (NON$\geq$ENR>SUP). (See Table 5b and Figure 10b.)

Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: GRAMS. There were no significant differences in the average amount of food consumed daily during Phase B. (See Table 5c and Figure 10a.)

Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: CALORIES. Animals in the enriched conditions ate less than animals housed in the non-enriched conditions and the difference between housing conditions approached significance during Phase B (0.068). (See Table 5d and Figure 10b.)

Specific Food Types examined separately:

Phase B – Standard Chow: GRAMS. There was a significant main effect for housing ($\underline{F}$ (2, 33) = 3.767, $\underline{p} < 0.05$) (NON<ENR<SUP). A Tukey HSD comparison revealed animals housed in Super-Enriched conditions ate significantly more food than animals in the Non-Enriched condition but that there were no significant differences in the amount of standard chow eaten between animals raised in Super-Enriched housing and Enriched or between animals raised in the Non-Enriched and Enriched conditions (**NON**<ENR<**SUP**). (Note: Bold type indicates groups that were significantly different.) (See Table 5e and Figure 10c.)

Phase B – Standard Chow: CALORIES. There was a significant main effect for housing ($\underline{F}$ (2, 33) = 3.767, $\underline{p} < 0.05$). A Tukey HSD comparison revealed that animals housed in Super-Enriched ate significantly more standard chow calories than animals in the Non-Enriched condition but that there were no significant differences in the amount of standard chow eaten between animals

raised in the Non-Enriched and Enriched conditions (**NON**<ENR<**SUP**). (Note: Bold type indicates groups that were significantly different.) (See Table 5f and Figure 10d.)

Phase B – Cookies: GRAMS. There were no significant differences in the average amount of cookies consumed. (See Table 5g and Figure 10c.)

Phase B – Cookies: CALORIES. There were no significant differences in the cookie calories consumed (See Table 5h and Figure 10d.).

Phase B – Potato Chip: GRAMS. There were no significant differences in the average amount of potato chips consumed. (See Table 5i and Figure 10c.)

Phase B – Potato Chip: CALORIES. There were no significant differences in the caloric intake of potato chips. (See Table 5j and Figure 10d.)

Phase A2 – Standard Chow Only Period: GRAMS. There were no significant differences in the amount of standard chow consumed during Phase A2. (See Table 5k and Figure 10a.)

Phase A2 – Standard Chow Only Period: CALORIES. There were no significant differences in the average number of calories consumed during Phase A2. (See Table 5l and Figure 10b.)

Physical Activity (PA)

Home Cage Activity (HCA). HCA was measured 17 times during Experiment I (six times during Phase B and 11 times during Phase A2). The average number of animals moving, amount of activity, and level of activity were observed during each measurement. Each type of HCA is presented for each experimental phase.

Phase B – Standard Chow with Sweet and Salty Foods Period. The Kruskal-Wallis Chi-Square revealed significant differences for number of animals moving, amount of activity, and level of activity. Kruskal-Wallis mean rank orders indicated that the enrichment conditions had the most animals moving: NON<ENR<SUP; the most amount of home cage activity: NON < ENR < SUP; and higher levels of activity: NON<ENR<SUP. (See Table 6a and Figures 11a – 11c.)

Phase A2 – Standard Chow Only Period. The Kruskal-Wallis Chi-Square was significant for number of animals moving, amount of activity, and level of activity. Kruskal-Wallis mean rank orders indicated that the enrichment conditions had the most animals moving: NON<SUP<ENR; the most amount of home cage activity: NON<ENR<SUP; and higher levels of activity: NON<ENR<SUP. (See Table 6b. and Figures 11a. - 11c.)

Open Field (OF). A total of six OF trials examining horizontal, vertical, and center-time activity were conducted during Experiment 1 (two per experimental phase A1, B, and A2). Overall MANOVAs revealed significant differences between housing conditions in total horizontal activity and vertical activity. Therefore, only the differences in horizontal and vertical activity were then examined using repeated-measures ANOVAs for each individual Open-Field trial. The results of the between-groups and the within-session analyses are presented separately for each Open-Field trial. Tables 7a – 7l present the details of the statistical analyses for the Open-Field trials. Figures 12a - 12d

present graphical depictions of the within-session horizontal and vertical activity for Open-Field trials.

<u>Phase A1 – Standard Chow Only Period, Open-Field Trial 1 Between Groups.</u> Horizontal activity (F (2,33) = 7.716, p < 0.05) and vertical activity (F (2,33) = 3.362, p < 0.05) were significantly different between housing conditions. Post Hoc pairwise comparisons revealed significantly lower horizontal activity in the SUP compared to ENR and NON (NON>ENR$\geq$SUP). Although between-subjects effects indicated a significant difference for vertical activity (F (2,33) = 3.362, p < 0.05), the post hoc pairwise analyses revealed no significant differences between any of the three housing conditions. (See Tables 7b & 7c and Figures 12a – 12d.)

<u>Phase A1 – Standard Chow Only Period, Open-Field Trial 1 Within Groups Within Session.</u> Repeated-measures ANOVAs revealed significant main effects for time (within session) on horizontal activity ($\underline{F}$ (11, 363) = 30.459, $\underline{p}$ < 0.05) and vertical activity ($\underline{F}$ (11, 363) = 14.704, $\underline{p}$ < 0.05) indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity ($\underline{F}$ (22, 363) = 3.826, $\underline{p}$ < 0.05) and vertical activity ($\underline{F}$ (22, 363) = 2.504, $\underline{p}$ < 0.05) indicated that the decrease in activity over time was different between the three housing conditions. Post Hoc pairwise comparisons revealed that the SUP housing condition activity was significantly lower than ENR, and NON (NON$\geq$ENR>SUP). (See Tables 7b & 7c and Figures 12a – 12d.)

Phase A1 – Standard Chow Only Period, Open-Field Trial 2 Between Groups. Horizontal activity (F (2,33) = 20.947, $p < 0.05$) and vertical activity (F (2,33) = 14.120, $p < 0.05$) were significantly different between housing conditions. Post Hoc pairwise comparisons revealed that the SUP housing condition was significantly different from the ENR and NON housing conditions (NON≥ENR>SUP). (See Tables 7d & 7e and Figures 12a – 12d.)

Phase A1 – Standard Chow Only Period, Open-Field Trial 2 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 363) = 47.499, $p < 0.05$) and vertical activity (F (11, 363) = 39.802, $p < 0.05$) indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity (F (22, 363) = 6.348, $p < 0.05$) and vertical activity (F (22, 363) = 5.035, $p < 0.05$) indicated that the decrease in activity over time was different between the three housing conditions. Post Hoc pairwise comparisons revealed that the amount of SUP animal's activity was significantly different from the ENR and the NON housed animals (NON≥ENR>SUP). (See Tables 7d & 7e and Figures 12a – 12d.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 3 Between Groups. Horizontal activity (F (2,33) = 13.394, $p < 0.05$) and vertical (F (2,33) = 8.966, $p < 0.05$) were significantly affected by housing condition. Post Hoc pairwise comparisons revealed that the SUP condition was significantly different from the ENR and NON housing conditions (NON≥ENR>SUP). (See Tables 7f & 7g and Figures 12a – 12d.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 3 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 363) = 47.521, $p < 0.05$) and vertical activity (F (11, 363) = 40.548, $p < 0.05$), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity (F (22, 363) = 4.47, $p < 0.05$) and vertical activity (F (22, 363) = 2. 408, $p < 0.05$), indicated that the decrease in activity over time was different between the three housing conditions. Post Hoc pairwise comparisons revealed that the amount of activity was significantly lower in the SUP compared to the ENR and the NON housed animals (NON≤ENR<SUP). (See Tables 7f & 7g and Figures 12a – 12d.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 4 Between Groups. Horizontal activity (F (2,33) = 9.604, $p < 0.05$) and vertical activity (F (2,33) = 6.186, $p < 0.05$) were significantly different between groups. Post Hoc pairwise comparisons revealed significantly lower activity for SUP and ENR compared to NON (NON>ENR≥SUP). (See Tables 7h & 7i and Figures 12a – 12d.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 4 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 363) = 85.290, $p < 0.05$) and vertical activity (F (11, 363) = 53.655, $p < 0.05$), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity (F (22, 363) = 4.475, $p < 0.05$)

and vertical activity (F (22, 363) = 2.693, $p < 0.05$) indicated that the decrease in activity over time was different between the three housing conditions. Post Hoc pairwise comparisons revealed that the amount of activity was lowest in the SUP followed by the ENR, and the NON housed animals had the greatest activity (NON<ENR<SUP). (See Tables 7h & 7i and Figures 12a – 12d.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 5 Between Groups. Horizontal activity (F (2,33) = 24.785, $p < 0.05$) and vertical activity (F (2,33) = 13.373, $p < 0.05$) were significantly different. Post Hoc pairwise comparisons revealed significant differences between all three groups for horizontal activity (NON>ENR>SUP) and no differences between the SUP and ENR groups for vertical activity (NON>ENR=SUP). (See Tables 7j & 7k and Figures 12a – 12d.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 5 Within Groups. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 363) = 80.477, $p < 0.05$) and vertical activity (F (11, 363) = 60.300, $p < 0.05$) indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity (F (22, 363) = 3.057, $p < 0.05$) and vertical activity (F (22, 363) = 2.763, $p < 0.05$) indicated that the decrease in activity over time was different between the three housing conditions. Post Hoc pairwise analyses revealed that the amount of activity was lowest in the SUP followed by the ENR, and the NON housed animals had the most activity (NON<ENR<SUP). (See Tables 7j & 7k and Figures 12a – 12d.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 6 Between Groups. Horizontal activity ($\underline{F}$ (2,33) = 16.079, $\underline{p} < 0.05$) and vertical activity ($\underline{F}$ (2,33) = 11.735, $\underline{p} < 0.05$) were significantly different between groups. Post Hoc pairwise comparisons revealed lower horizontal and vertical activity for SUP and ENR compared to NON (NON<ENR$\leq$SUP). (See Tables 7l & 7m and Figures 12a – 12d.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 6 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($\underline{F}$ (11, 363) = 72.093, $\underline{p} < 0.05$) and vertical activity ($\underline{F}$ (11, 363) = 41.351, $\underline{p} < 0.05$), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity ($\underline{F}$ (22, 363) = 3.840, $\underline{p} < 0.05$) and vertical activity ($\underline{F}$ (22, 363) = 2.230, $\underline{p} < 0.05$) indicated that the decrease in activity over time was different for the three housing conditions. Post Hoc pairwise comparisons revealed that the amount of activity was lowest for the SUP followed by the ENR, and the NON housed animals had most activity (NON<ENR<SUP). (See Tables 7l & 7m and Figures 12a – 12d.)

Open-Field Results Summary. Animals in the enriched housing conditions were less active during each of the six OF trials compared to the non-enriched condition. The difference between the three housing conditions indicated that the animals housed in the NON condition moved the most, followed by ENR, and the SUP housed animals moved the least. These differences in activity in the housing conditions are consistent with other studies

(Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996; Varty et al., 2000; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). (See Figures 12a – 12d for graphical representations of averaged activity.)

Discussion: Experiment I

Experiment I examined the effects of environmental enrichment on body weight, feeding, and activity in male, adolescent, Sprague-Dawley rats. This experiment was designed to examine the effects of environmental enrichment on: (1) body weights when the different foods are available, (2) food consumption, using bland foods and other, more preferred foods, and (3) physical activity, including activity in an open field arena and within the home cage.

Environmental enrichment was associated with lower body weights over the seven-week experiment. This finding is consistent with previous research (e.g., Sclafani & Springer, 1976; Tomchesson, 2004). Additionally, animals housed in enriched conditions had significantly lower BMI and LI scores at the end of the experiment compared to the animals in the non-enriched housing. These lower indices suggest that animals housed in enriched environments had lower amounts of body fat (Maffie et al., 1995).

Enrichment appeared to decrease the amount of food consumed for standard chow early in the experiment. This decreased food intake would be consistent with lower body weights and body fat. The decreased feeding became less marked as different types of foods (sweet & salty) were made available, as time went by, and as the subjects aged. Clearly, the amount of standard chow consumed decreased when the cookies and chips were offered,

but there were no significant differences between housing conditions in the amount of standard chow or potato chips eaten during Phase B.

Physical activity was measured in novel open field environments and in home cages. Open Field activity (horizontal and vertical activity) was lower for the animals housed in enrichment and was the lowest for the animals housed in the SUP condition. The effect for enrichment to decrease OF activity is consistent with previous reports (e.g., O'Conner & Eikelboom, 2000; Varty et al., 2000; Elliott 2004; Tomchesson, 2004). This effect for enrichment to decrease activity in the OF is not consistent with a decrease in body weight or body fat. It is consistent with effects of enrichment on learning (decreases in OF activity may indicate improved habitation a simple form of learning) (Varty et al., 2000). Conversely, animals in enriched environments exhibited more activity in the home cages compared to animals in non-enriched housing. The increased amount and intensity (i.e., level) of activity in the home cage is consistent with decreases in body weight. Further, home cage activity was consistent with the differential decreases in body weight seen in the different housing conditions (i.e., BW = NON>ENR≥SUP and HCA = NON<ENR≤SUP). The findings from Experiment I examining three different types of environmental enrichment with varying amounts of physical and social activity suggest an increased effect of increased levels of environmental enrichment. Specifically, more opportunities for physical and social stimulation resulted in higher amounts and levels of activity in the home cage and lower body weights. It is clear from Experiment I that environmental enrichment decreased body weight, had a small effect on

feeding, and led to increased activity in the home cage for male rats. However, it was not clear if there were differences in the amounts of the different types of foods eaten when the animals had a choice because the difference between housing conditions in the amount of cookies eaten did not reach significance.

Experiment II

Experiment II examined the effects of environmental enrichment on body weight, feeding, and activity (open-field activity and home cage activity) in female, adolescent, Sprague-Dawley rats. Experiment II was conducted using the procedures of Experiment I except that the subjects were adolescent female rats. The “group activity” technique to evaluate home cage activity that was developed during Experiment I was used for this experiment. A detailed description of “group activity” is provided in the methods section of Experiment I.

Identical to Experiment I, the goals of Experiment II were to determine the effects of environmental enrichment on: (1) body weight when different types of foods (sweet and salty) are available, (2) food consumption (bland foods and other foods), and (3) physical activity (open field arena and home cage).

Hypotheses: Experiment II

Hypothesis 1 – EE, BW, BMI, and LI

Environmental enrichment will decrease body weight gains and body mass index such that: Non-Enriched > Enriched > Super-Enriched.

Rationale. Environmental enrichment has been reported to decrease the rate of weight gain in rats (O’Conner & Eikelboom, 2000; Hellemans, Benge, Olmstead, 2004; Tomchesson, 2004).

Hypothesis 2 – EE and FC

A) Environmental enrichment will decrease the amount of bland foods consumed compared to animals reared in Non-Enrichment such that: Non-Enriched > Enriched > Super-Enriched.

Rationale. Rats housed in environmental enrichment eat less bland food (Sclafani & Springer, 1976; Tomchesson, 2004).

B) Animals raised in enriched and non-enriched housing conditions will eat similar amounts of cafeteria foods such that: Non-Enriched = Enriched = Super-Enriched.

Rationale. Rats housed in environmental enrichment did not eat less cafeteria food than singly housed controls (Sclafani & Springer, 1976).

Hypothesis 3 – EE and PA

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Non-Enriched > Enriched > Super-Enriched.

Rationale. Enriched animals reduced locomotor activity and reduced exploration over time compared to non-enriched animals (van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001; Varty et al., 2000).

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Non-Enriched < Enriched < Super-Enriched.

Rationale. Animals exposed to longer activity periods have higher daily energy expenditure (Boon, Visser, Daan, 1997) and animals that are allowed access to exercise (i.e., a running wheel) choose exercise over feeding (Premack & Premack, 1963; Boakes & Dwyer, 1997; Mueller, Herman, & Eikelboom, 1999; Lattanzio & Eikelboom, 2003). Therefore, animals in the enriched housing conditions, that have more opportunity for physical activity, will be more active.

Methods: Experiment II

The methods and design of Experiment I were used in Experiment II with few modifications. Specifically, female subjects were used and home cage activity was measured using the group activity measure only.

Subjects

Subjects were 36, adolescent (21 days old on arrival), female Sprague-Dawley rats, resulting in 12 subjects per cell.

Housing

Housing conditions were identical to Experiment I. All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001 or "Junk" Foods) and water. Animals were assigned to one of three housing conditions (NON, ENR, or SUP) as described in Experiment I.

Procedures

The procedures in Experiment II were the same as in Experiment I with one exception. Only the group activity measure was used to measure home cage activity. This change was made because during Experiment I, measuring

group activity provided a more accurate and logistically feasible measurement of physical activity than did the individual activity measure. A detailed description of the "group activity" measure and rationale is provided in the methods of Experiment I. (See Table 8 in Section IV.)

Dependent Variables

The dependent variables were identical to Experiment I.

Body Weight (BW). BW was measured on Day 1 and then three times during each two-week experimental phase (the 5th, 10th, and 14th day) and two additional times for Phase A2 (the 18th and 21st day).

Body Mass Index (BMI). BMI was measured on the final day of the experiment.

Lee Index (LI). LI was measured on the final day of the experiment.

Food Consumption (FC). Food weights were measured every other day providing a total of 21 Food Consumption measurements.

Physical Activity (PA). *Home Cage Activity – Group Activity.* Group activity was observed a minimum of five times during experimental phase B and A2 only.

Open-Field (OF). OF activity was measured at the beginning and end of each experimental phase (Days A1-4, A1-13, B1-3, B1-13, A2-3, and A2-20).

Results: Experiment II

Body Weight (BW)

Body weight was measured 12 times during the experiment (four times during Phase A1, three times during Phase B, and five times during the Phase

A2). An overall ANCOVA revealed a main effect for Time (F (10, 320) = 12.554, $p < 0.05$) and a main effect for housing (F (2, 32) = 7.827, $p < 0.05$), and a Time X Housing interaction (F (10, 320) = 3.082, $p < 0.05$). Post-hoc pairwise comparisons using a Bonferroni adjustment for multiple comparisons revealed that the body weight of animals in the Super-Enriched condition was less than the Enriched and the Non-Enriched conditions. Additionally, animals housed in Super-Enriched cages weighed significantly less than animals housed in Non-Enriched cages, but the differences in the weights of animals housed in Non-Enriched and Enriched housing were not significant (NON≥ENR>SUP). There were no significant differences in body length between the three groups (See Table 22). (See Table 9 and Figures 13a -13c for Body Weight results.)

Body Mass Index (BMI)

A significant main effect for housing (F (2, 33) = 6.836, $p < 0.05$) and Tukey HSD post hoc analyses revealed that animals in the SUP housing condition had significantly lower average BMIs compared to animals raised in the ENR condition and NON housing. Animals in the ENR and NON conditions were not significantly different (NON≥ENR>SUP). (See Tables 10 and Figure 14.)

Lee Index (LI)

A significant main effect for housing (F (2, 33) = 4.342, $p < 0.05$) and Tukey HSD post hoc analyses revealed that animals in the SUP housing condition had significantly lower average LI's than the animals raised in the ENR condition and the NON conditions (NON≥ENR>SUP). (See Table 11 and Figure 15.)

Food Consumption (FC)

Food consumption (FC) was measured 25 times during Experiment I (seven measurements for Phase A1 & B and 11 measurements for Phase A2). Average grams and calories consumed daily were examined separately for each experimental phase because the available foods differed for each phase (i.e., only standard chow was available during Phases A1 & A2 and standard chow, salty, and sweet foods were available during Phase B). The results for grams and calories are presented separately for each phase of the experiment.

Phase A1 – Standard Chow Only Period: GRAMS. There was a significant main effect for housing ($\underline{F}$ (2, 33) = 3.835, $\underline{p} < 0.05$). Tukey HSD post-hoc comparisons revealed that animals housed in Super-Enriched conditions and in Enriched conditions ate significantly fewer calories than did animals in the Non-Enriched housing but there was no difference between animals housed in Super-Enriched and Enriched conditions (NON>SUP$\geq$ENR). (See Table 12a and Figure 16a.)

Phase A1 – Standard Chow Only Period: CALORIES. There was a significant main effect for housing ($\underline{F}$ (2, 33) = 3.767, $\underline{p} < 0.05$). Tukey HSD post-hoc comparisons revealed that animals housed in Super-Enriched conditions and in Enriched conditions ate significantly fewer calories than did animals in the Non-Enriched housing, but there was no difference between animals housed in Super-Enriched and Enriched conditions (NON>SUP$\geq$ENR). (See Table 12b and Figure 16b & 16c.)

Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: GRAMS. There were no significant differences. (See Table 12c and Figure 16c.)

Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: CALORIES. There were no significant differences. (See Table 12d and Figure 16d.)

Phase B – Standard Chow: GRAMS. There were no significant differences. (See Table 12e and Figure 16c.)

Phase B – Standard Chow: CALORIES. There were no significant differences. (See Table 12f and Figure 16d.)

Phase B – Cookies: GRAMS. There was a significant difference in the average amount of cookies consumed (F (2, 33) = 13.899, p < 0.05). Super-Enriched housed animals ate significantly less cookies than Enriched housed animals, that ate significantly less than animals housed in the Non-Enriched conditioned (NON>ENR>SUP). (See Table 12g and Figure 16c.)

Phase B – Cookies: CALORIES. There was a significant difference in the average number of cookie calories consumed (F (2, 33) = 13.899, p < 0.05). Super-Enriched housed animals ate significantly less cookie calories than Enriched housed animals, that ate significantly less cookie calories than animals housed in the Non-Enriched conditioned (NON>ENR>SUP). (See Table 12h and Figure 16d.)

Phase B – Potato Chip: GRAMS. There were no significant differences. (See Table 12i and Figure 16c.)

Phase B – Potato Chip: CALORIES. There were no significant differences. (See Table 12j and Figure 16d.)

Phase A2 – Standard Chow Only Period: GRAMS. There were no significant differences. (See Table 12k and Figure 16a.)

Phase A2 – Standard Chow Only Period: CALORIES. There were no significant differences. (See Table 12l and Figure 16b.)

Physical Activity (PA)

Home Cage Activity (HCA). HCA was measured 17 times during Experiment II (five times during Phase A1, five times during Phase B, and seven times during Phase A2). The average number of animals moving, amount of activity, and level of activity were observed during each measurement. Each type of HCA is presented for each experimental phase.

<u>*Phase A1 – Standard Chow Only Period.*</u> The Kruskal-Wallis Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity. Kruskal-Wallis mean rank orders indicated that all enrichment conditions were similar with regard to the mean number of animals moving NON=ENR=SUP, but that animals in the SUP condition had the most activity: NON<ENR<SUP, and had higher levels of activity: NON<ENR<SUP compared to animals in the other housing conditions. (See Table 13a and Figures 17a - 17c.)

<u>*Phase B – Standard Chow with Sweet and Salty Foods Period*</u>. The Kruskal-Wallis Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity. Kruskal-Wallis mean

rank orders indicated that all enrichment conditions had the same rank order with regard to the mean number of animals moving: NON=ENR=SUP, but that animals in the SUP condition moved the most: NON<ENR<SUP, and had the highest levels of activity: NON<ENR<SUP. (See Table 13b and Figures 17a - 17c.)

Phase A2 – Standard Chow Only Period. The Kruskal-Wallis test Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity. Kruskal-Wallis mean rank orders indicated that all enrichment conditions had the same rank order with regard to the mean number of animals moving: NON=ENR=SUP, but that animals in the SUP condition moved the most: NON<ENR<SUP, and had the highest levels of activity: NON<ENR<SUP. (See Table 13c and Figures 17a - 17c.)

Open Field (OF). A total of six OF trials examining horizontal, vertical, and center-time activity were accomplished during Experiment II (2 per experimental phase; A1, B, and A2). Overall MANOVAs revealed significant differences between housing conditions in total horizontal activity or vertical activity (See Table 12a). Therefore, the differences in horizontal and vertical activity were then examined using repeated-measures ANOVAs for each individual Open-Field trial. The results of the between-groups and the within-session analyses are presented separately for each Open-Field trial. Tables 14a – 14i present the details of the statistical analyses for the Open-Field trials. Figures 18a - 18d present graphical depictions of the within-session horizontal and vertical activity for Open-Field trials.

Phase A1 – Standard Chow Only Period, Open-Field Trial 1 Between Groups. There were significant differences for horizontal activity (F (2,33) = 21.539, $p < 0.05$) and vertical activity (F (2,33) = 13.313, $p < 0.05$). Pairwise comparison post hoc analyses revealed that animals in the SUP condition moved significantly less than the ENR and NON conditions. Further, there were no significant differences between ENR and NON housed animals for horizontal and vertical activity NON$\leq$ENR<SUP. (See Tables 14b & 14c and Figures 18a & 18b.)

Phase A1 – Standard Chow Only Period, Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 363) = 63.488, $p < 0.05$) and vertical activity (F (11, 363) = 48.803, $p < 0.05$). Animals' horizontal and vertical activity were different over time. In addition, there was a significant time by housing interaction for horizontal activity (F (22, 363) = 1.911, $p < 0.05$) and vertical activity (F (22, 363) = 2.029, $p < 0.05$). Post hoc analyses indicated that over time, SUP housed animals moved the least, followed by the ENR, and the NON housed animals had the highest amount of activity (NON<ENR<SUP). (See Tables 14b & 14c and Figures 18a & 18b.)

Phase A1 – Standard Chow Only Period, Open-Field Trial 2 Between Groups. Horizontal activity (F (2,33) = 47.870, $p < 0.05$) and vertical activity (F (2,33) = 28.744 $p < 0.05$) were significantly different between housing conditions. Pairwise comparisons indicated that SUP horizontal was significantly less than ENR, which was significantly less than NON housing conditions

(NON>ENR>SUP). Animals in SUP conditions had significantly less vertical activity than did animals in the ENR and NON conditions, which were not significantly different from each other: NON≥ENR>SUP. (See Tables 14d & 14e and Figures 18a & 18b.)

Phase A1 – Standard Chow Only Period, Open-Field Trial 2 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 363) = 49.272, p < 0.05) and vertical activity (F (11, 363) = 28.744, p < 0.05), indicating that horizontal and vertical activity were different over time. In addition, there was a significant time by housing interaction for horizontal activity (F (22, 363) = 1.863, p < 0.05) and vertical activity (F (22, 363) = 1.807, p < 0.05). Post hoc analyses indicated that the decrease in activity over time was different for the three housing conditions such that the amount of activity decrease was most in the SUP followed by the ENR, and the NON housed animals had the lowest activity (NON<ENR<SUP). (See Tables 14d & 14e and Figures 18a & 18b.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 3 Between Groups. Horizontal activity (F (2,33) = 11.052, p < 0.05) was significantly different between groups. Post-hoc analyses revealed that horizontal activity was lower for SUP compared to ENR and NON (NON≥ENR>SUP). There were no significant differences in vertical activity. (See Tables 14f & 14g and Figures 18a & 18b.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 3 Within Groups Within Session. Repeated-measures ANOVAs

revealed significant main effects for time on horizontal activity ($\underline{F}$ (11, 363) = 44.923, $\underline{p} < 0.05$) and vertical activity ($\underline{F}$ (11, 363) = 33.911, $\underline{p} < 0.05$), indicating that horizontal and vertical activity were different over time. A significant time by housing interaction for horizontal activity ($\underline{F}$ (22, 363) = 1.170, $\underline{p} < 0.05$) suggests that animal horizontal activity decreased differently over time in all conditions. Post hoc analyses revealed that the decrease in horizontal activity over time was different between the three housing conditions such that activity decreased most in the SUP followed by the ENR, and the NON housed animals had the lowest amount of activity (NON<ENR<SUP). There was no significant time by housing interaction for vertical activity. (See Tables 14f & 14g and Figures 18a & 18b.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 4 Between Groups. Horizontal activity ($\underline{F}$ (2,33) = 28.730, $\underline{p} < 0.05$) and vertical activity ($\underline{F}$ (2,33) = 7.744, $\underline{p} < 0.05$) was significantly different between groups. Post Hoc Pairwise comparisons revealed lower activity for SUP and ENR compared to NON, but no significant difference between ENR and NON (NON$\geq$ENR>SUP). (See Tables 14h & 14i and Figures 18a & 18b.).

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 4 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity ($\underline{F}$ (11, 363) = 60.282, $\underline{p} < 0.05$) and vertical activity ($\underline{F}$ (11, 363) = 32.367, $\underline{p} < 0.05$), indicating that horizontal and vertical activity were different over time. Significant time by housing interactions for horizontal activity ($\underline{F}$ (22, 363) = 1.807, $\underline{p} < 0.05$) and

vertical activity (F (22, 363) = 1.810, $p < 0.05$) indicate that the difference in activity was different for the various housing groups. Post Hoc analyses revealed the decrease in activity over time was different for the three housing conditions such that the SUP housed animals moved the least, followed by the ENR, and the NON housed animals had the most activity (NON<ENR<SUP). (See Tables 14h &14i and Figures 18a & 18b.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 5 Between Groups. Horizontal activity (F (2,33) = 25.648, $p < 0.05$) and vertical activity (F (2,33) = 12.984, $p < 0.05$) were significantly different between groups. Post Hoc analyses revealed that horizontal and vertical activity were significantly lower animals in SUP housing compared to animals in the ENR, and animals in the ENR housing compared to animals in the NON housing (NON>ENR>SUP). (See Tables 14j & 14k and Figures 18a & 18b.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 5 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 363) = 67.661, $p < 0.05$) and vertical activity (F (11, 363) = 42.938, $p < 0.05$), indicating that animal activity decreased over time in all conditions. In addition, there was a significant time by housing interaction for horizontal activity (F (22, 363) = 1.596, $p < 0.05$) and vertical activity (F (22, 363) = 1.695, $p < 0.05$). Post Hoc analyses revealed that the over time, the amount of activity was least in the SUP followed by the ENR, and the NON housed animals had the most activity (NON<ENR<SUP). (See Tables 14j &14k and Figures 18a & 18b.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 6 Between Groups. Horizontal activity (F (2,33) = 7.528, $p < 0.05$) and vertical activity (F (2,33) = 6.497, $p < 0.05$) were significantly different between groups. Post Hoc Pairwise comparisons indicated lower activity for SUP housed animals compared to animals housed in the ENR and NON conditions. There were no significant differences between the animals in the ENR and NON housing conditions. For both horizontal and vertical activity: NON≥ENR>SUP. (See Tables 14l & 14m and Figures 18a & 18b.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 6 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 363) = 56.915, $p < 0.05$) and vertical activity (F (11, 363) = 37.761, $p < 0.05$), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity (F (22, 363) = 1.740, $p < 0.05$) and vertical activity (F (22, 363) = 1.468, $p < 0.05$) indicated that the decrease in activity over time was different for the three housing conditions. Post Hoc Pairwise comparisons revealed that the amount of activity was least in the SUP followed by the ENR, and the NON housed animals had the most activity: NON≥ENR>SUP. (See Tables 14l & 14m and Figures 18a & 18b.)

Open-Field Results Summary. Animals in the Enriched housing conditions were less active during each of the six OF trials. These animals had the greatest decrease in activity within each OF session compared with animals in the Non-Enriched housing conditions. (See Figures 18a – 18d.) In particular,

the animals in the Super-Enriched condition moved less than animals in the Enriched condition, which moved less than animals in the Non-Enriched condition. These differences in activity in the housing conditions are consistent with other studies (Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996; Varty et al*.,* 2000; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). Further, there was a difference between all three housing conditions in the amount of activity between OF trials such that the animals housed in the NON condition moved the most and followed by ENR, and the SUP housed animals moved the least. These effects were consistent with the findings from Experiment I of this investigation.

Discussion: Experiment II

Experiment II examined the effects of environmental enrichment on activity, feeding, and body weight in female, adolescent, Sprague-Dawley rats. Like Experiment I, this experiment was designed to examine the effects of environmental enrichment on; (1) body weight when the different foods are available, (2) food consumption, using bland foods and other, more preferred foods, and (3) physical activity, including activity in an open field arena and within the home cage.

The results of Experiment II indicated that animals housed in environmental enrichment had significantly lower body weights. This finding is consistent with Experiment I and with previous research (e.g., Sclafani & Springer, 1976; Tomchesson, 2004). Additionally, animals housed in the Super-Enrichment condition had significantly lower BMI and LI scores compared to the

Enriched and Non-Enriched conditions. These lower indices suggest that animals housed in enriched environments had lower amounts of body fat, particularly the SUP housed animals (Maffie et al., 1995). There were no signs of ill health in the animals that might account for the lower BW, BMI, or LI.

As in Experiment I, enrichment again decreased the amount of standard chow eaten early in the Experiment II. As before, the initial decreased feeding was consistent with lower body weights and body fat indices. However, during Phase B, the consumption of all foods again increased. There were no differences in the standard chow or potato chips eaten between housing groups. There was a significant difference in the amount of cookies eaten, with the Non-Enriched housed animals eating significantly more than the Enriched housed and Super-Enriched housed animals. In addition, during Phase A2, it appeared that the Super-Enriched began eating more bland food than both other groups, although this difference was not statistically significant.

Physical activity was measured in novel open field environments and in home cages. Open Field activity (horizontal and vertical activity) was lower for the animals housed in enrichment and was the lowest for the animals housed in the SUP condition. The effect for enrichment to decrease OF activity is consistent with Experiment I and previous reports (e.g., O'Conner & Eikelboom, 2000; Varty et al., 2000; Elliott 2004; Tomchesson, 2004). The decrease in OF activity is not consistent with lower body weights or body fat indices. Animals in the environmental enrichment were more active in their home cages, consistent with decreased body weight. Further, the home cage activity scores were

consistent with the differential decreases in body weight seen in the different housing conditions (i.e., $\text{BW} = \text{NON} > \text{ENR} \geq \text{SUP}$ and $\text{HCA} = \text{NON} < \text{ENR} \leq \text{SUP}$).

Given the findings from Experiments I & II, it was important to attempt to replicate the decreased body weight and increased home cage activity in male and female subjects. In addition, it was also necessary to examine male and female subjects together to directly compare any gender differences. Interestingly, food consumption findings from this experiment suggest possible gender differences in the types of foods consumed by Enriched and Non-Enriched housed animals. Specifically, females in Non-Enriched housing ate significantly more cookies than did the females in the enriched conditions. This same difference did not reach significance for males, although there was a trend in this direction, in Experiment I.

Experiment III

Experiment III was conducted to attempt to replicate the findings of Experiments I and II and to allow a direct comparison of male and female subjects within the same experiment. The exact procedures of Experiment III were determined based on the results of the first two experiments. Experiment III was a 2 (housing condition) X 2 (gender) repeated-measures design investigating the effects of environmental enrichment on food consumption, physical activity, and body weight in male and female adolescent rats. The 7-week experiment used the same food types (sweet with bland, salty with bland, or bland only) and the housing conditions (NON, SUP) that had the greatest effects on body weight in the first two experiments. The findings from the FC

data regarding specific foods were unclear because in Experiment I, there was a trend for cookie consumption, but the difference was not statistically significant approaching significance. Conversely, the difference in cookie consumption among housing conditions was statistically significant in Experiment II. It is possible that there is a gender difference in the types of specific foods consumed, however, it is not possible to make a direct comparison, as these were two distinct and separate experiments. HCA data were clear but the measure was new and replicating the results is important to establish the reliability of the measure. The goals of Experiment III were to: (1) compare the effects of environmental enrichment on body weight in male and female adolescent rats, (2) compare the effects of environmental enrichment on food consumption in male and female adolescent rats, and (3) compare the effects of environmental enrichment on physical activity in male and female adolescent rats. The individual hypotheses for Experiment III were based on the results of Experiments I and II.

Hypotheses: Experiment III

Hypothesis 1 – EE, BW, BMI, and LI

Environmental enrichment will decrease body weight gains and body mass index such that: Non-Enriched > Super-Enriched.

Hypothesis 2 – EE and FC

A) The animals in the Super-Enrichment condition will consume less bland food than Non-Enriched housing.

B) Males will consume more standard rat chow than females.

C) Males and females will consume more food during Phase B compared to Phase A1 & A2 regardless of housing condition.

D) There will be a sex by housing interaction for the type of foods consumed during Phase B for females such that animals reared in the Super-Enriched condition will eat less than animals in the Non-Enriched condition during Phase B. However, there will be no significant differences in the amount of food consumed by males during Phase B.

Hypothesis 3 – EE and PA

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Non-Enriched > Super-Enriched.

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Non-Enriched < Super-Enriched.

C) Males and females will exhibit different activity levels in both Open-Field trials and Home Cage observations such that males will be less active in the Open-Field, but more active in the home cage.

Methods: Experiment III

The methods and design of Experiments I and II were used in Experiment III with three main differences. First, both male and female subjects were used in an attempt to replicate the findings of the first two experiments and to allow for a direct gender comparison. Second, as in Experiment II, only the group home cage activity (HCA) method was used. Third, Open-Field (OF) activity was

measured three times at the end of each experimental phase and not six times at the beginning and end of each phase as in the previous experiments. This change was made because of the robust and consistent findings of previous experiments, including the first two experiments in this project.

Subjects

Subjects were 48, adolescent (21 days old on arrival), male (24) and female (24) Sprague-Dawley rats.

Housing

Housing conditions were identical to Experiments I & II with the exception that only the Non–Enriched and Super-Enriched housing conditions were used. The use of these two housing conditions was determined by the results of the first two experiments. All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001 or “Junk” Foods) and water. Two Super-Enriched housing cages were used in this experiment. Each cage housed one sex of rats.

Procedures

The procedures in Experiment III were virtually identical to Experiments I and II. Experiment III was a 7-week, repeated-measures A-B-A design. Unlike the first two experiments, it was a 2 (housing condition) X 2 (sex) repeated-measures design. OF activity was measured only three times (not six) because the measure was so consistent that it proved to be redundant to measure it six times. (See Table 15 for Experiment III Timeline.)

Dependent Variables

The dependent variables were identical to Experiment I & II.

Body Weight (BW). BW was measured on Day 1 and then three times during each 14-day experimental phase (the 5^{th}, 10^{th}, and 14^{th} day) and two additional times for Phase A2 (the 18^{th} and 21^{st} day).

Body Mass Index (BMI). BMI was measured on the final day of the experiment.

Lee Index (LI). LI was measured on the final day of the experiment.

Food Consumption (FC). Food weights were measured every other day providing a total of 21 Food Consumption measurements.

Physical Activity (PA). *Home Cage Activity – Group Activity.* Group activity was observed a minimum of five times during experimental phase B and A2.

Open-Field (OF). OF activity was measured three times, once at the end of each experimental phase (Days, A1-13, B1-13, and A2-20).

Results: Experiment III

Body Weight (BW)

Body weight was measured 12 times during the experiment (four times during Phase A1, three times during Phase B, and five times during the Phase A2). A between-subjects main effect for housing (F (1, 42) = 5.578, p < 0.05) indicated that animals in the Non-Enriched housing weighed significantly more than animals in the Super-Enriched housing. A between-subjects main effect for sex (F (1, 42) = 15.293, p < 0.05) revealed that the males weighed significantly

more than females. Post Hoc pairwise comparisons revealed that males housed in Super-Enriched cages weighed significantly less than males housed in Non-Enriched cages, and females housed in Super-Enriched cages weighed significantly less than females in Non-Enriched housing (male NON > male SUP > female NON > female SUP). A main effect for Time revealed that body weights differed over time (F (10, 420) = 4.156, $p < 0.05$). A Time X Housing interaction (F (10, 420) = 12.096, $p < 0.05$) and a Time X Sex interaction (F (10, 420) = 116.116, $p < 0.05$) revealed that over time, animals weights differed when accounting for housing condition and sex. Post Hoc pairwise comparisons revealed that animals in the NON housing gained weight faster than animals in the SUP housing and males gained weight faster than females. There were no significant differences in body length between the housing conditions. There was a significant difference in body length between males and females, as would be expected given the difference in sizes between the males and females, and there was not a gender by housing interaction (See Table 22). (See Table 16 and Figures 19a -19c. for body weight results)

Body Mass Index (BMI)

Animals housed in Super-Enriched housing had significantly different BMIs compared to animals housed in Non-Enriched housing (F (1,43) = 15.458, $p < 0.05$). Females had significantly different BMI than males (F (1,43) = 14.674, $p < 0.05$). There was no housing X sex interaction. Tukey HSD post hoc analyses revealed a strong trend for animals in the Super-Enriched Females to have significantly lower BMIs compared to Non-Enriched Females. Super-

Enriched Males had significantly lower BMIs than Non-Enriched Males (NON Males> NON Females $\geq$ SUP Males $\geq$ SUP Females). (See Table 17 and Figure 20.)

Lee Index (LI)

Animals in Super-Enriched housing had significantly lower LIs compared to animals housed in Non-Enriched housing (F (1,43) = 8.190, $p < 0.05$). There was no main effect for sex, nor a sex X housing interaction. (NON Females $\geq$ NON Males $\geq$ SUP Females $\geq$ SUP Males). (See Table 18 and Figure 21.)

Food Consumption (FC)

Food consumption (FC) was measured 25 times during Experiment I (seven measurements for Phase A1 & B and 11 measurements for Phase A2). Average grams and calories consumed daily were examined separately for each experimental phase because the available foods differed for each phase (i.e., during Phases A1 & A2, only standard chow was available and during Phase B, standard chow, salty, and sweet foods were available). The results for grams and calories are presented separately for each phase of the experiment.

Phase A1 – Standard Chow Only Period: GRAMS. Animals in the SUP housing ate significantly less than animals housed in the NON housing (F (1, 43) = 69.175, $p < 0.05$) and females ate significantly less than males (F (1, 43) = 43.469, $p < 0.05$). A Tukey HSD comparison revealed Super-Enriched females ate significantly less than Super-Enriched males and Non-Enriched females that ate significantly less than Non-Enriched males (NON Males > NON Females $\geq$ SUP Males > SUP Females). (See Table 19a and Figure 22a.)

Phase A1 – Standard Chow Only Period: CALORIES. Animals in the SUP housing ate significantly fewer calories than animals housed in the NON housing ($\underline{F}$ (1, 43) = 69.175, $\underline{p} < 0.05$) and females ate significantly fewer calories than males ($\underline{F}$ (1, 43) = 43.469, $\underline{p} < 0.05$). A Tukey HSD comparison revealed Super-Enriched females ate significantly less than Super-Enriched males and Non-Enriched females who ate significantly less than Non-Enriched males (NON Males > NON Females $\geq$ SUP Males > SUP Females). (See Table 19b and Figure 22b.)

Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: GRAMS. Animals in the SUP housing ate significantly less than animals housed in the NON housing ($\underline{F}$ (1, 43) = 40.851, $\underline{p} < 0.05$) and females ate significantly less than males ($\underline{F}$ (1, 43) = 4.342, $\underline{p} < 0.05$). A Tukey HSD comparison revealed Super-Enriched females ate significantly less than Non-Enriched females, that ate similar amounts of chow compared to Super-Enriched males, that ate significantly less than Non-Enriched males (NON Males $\geq$ SUP Males $\geq$ NON Females > SUP Females). (See Table 19c and Figure 22a.)

Phase B – Standard Chow with Sweet and Salty Foods Period, All Foods: CALORIES. Animals in the SUP housing ate significantly fewer calories than animals housed in the NON housing ($\underline{F}$ (1, 43) = 40.851, $\underline{p} < 0.05$) and females ate significantly fewer calories than males ($\underline{F}$ (1, 43) = 4.342, $\underline{p} < 0.05$). A Tukey HSD comparison revealed Super-Enriched females ate only slightly fewer calories than Super-Enriched males, who ate significantly fewer calories than Non-Enriched females, who ate significantly fewer than Non-Enriched males

(NON Males > NON Females > SUP Males $\geq$ SUP Females). (See Table 19d and Figure 22b.)

Specific Food Types examined separately:

Phase B – Standard Chow: GRAMS. Animals in the SUP housing did not eat significantly less than animals housed in the NON housing, however, females ate significantly less than males (F (1, 43) = 150.316, $p < 0.05$). In addition, a significant housing by sex interaction (F (1, 43) = 4.999, $p < 0.05$) revealed that females housed in the SUP condition ate less standard chow than did females in the NON condition, but males in the SUP ate more standard chow than did NON housed males. A Tukey HSD comparison revealed that Super-Enriched females ate slightly less standard chow than Non-Enriched females, that ate significantly less than Super-Enriched males, that ate marginally less than Non-Enriched males (SUP Males $\geq$ NON Males > NON Females $\geq$ SUP Females). (See Table 19e and Figure 22c.)

Phase B – Standard Chow: CALORIES. Animals in the SUP housing did not eat significantly less than animals housed in the NON housing; however, females ate significantly less than males (F (1, 43) = 150.316, $p < 0.05$). In addition, a significant housing by sex interaction (F (1, 43) = 4.999, $p < 0.05$) revealed that female animals housed in the SUP condition ate less standard chow than did females in the NON condition, but males in the SUP ate more standard chow than did NON housed males. A Tukey HSD comparison revealed that Super-Enriched females ate slightly less standard chow than Non-Enriched females, that ate significantly less than Super-Enriched males, that ate

marginally less than Non-Enriched males (SUP Males ≥ NON Males > NON Females ≥ SUP Females). (See Table 19f and Figure 22d.)

Phase B – Cookies: GRAMS. Animals in the SUP housing ate significantly less than animals housed in the NON housing (F (1, 43) = 17.454, $p < 0.05$) and there was no difference in the amount of cookies consumed between males and females. A Tukey HSD comparison revealed Super-Enriched males ate slightly less cookies than Super-Enriched females, that ate less than Non-Enriched males, that ate less than Non-Enriched females (NON Females ≥ NON Males ≥ SUP Females ≥ SUP Males). (See Table 19g and Figure 22c.)

Phase B – Cookies: CALORIES. Animals in the SUP housing ate significantly fewer calories than animals housed in the NON housing (F (1, 43) = 25.938, $p < 0.05$) and there was no difference in the cookie calories consumed between males and females. A Tukey HSD comparison revealed that Super-Enriched males ate slightly less cookies than Super-Enriched females, that ate less than Non-Enriched males, that ate less than Non-Enriched females (NON Females ≥ NON Males ≥ SUP Females ≥ SUP Males). (See Table 19h and Figure 22d.)

Phase B – Potato Chip: GRAMS. Animals in the SUP housing ate significantly fewer potato chips than animals housed in the NON housing (F (1, 43) = 21.237, $p < 0.05$) and there was no difference in the amount of cookies consumed between males and females. A Tukey HSD comparison revealed that Super-Enriched males ate slightly fewer cookies than Super-Enriched females, that ate less than Non-Enriched males, that ate less than Non-Enriched females

(NON Males ≥ NON Females > SUP Males ≥ SUP Females). (See Table 19i and Figure 22c.)

Phase B – Potato Chip: CALORIES. Animals in the SUP housing ate significantly fewer chip calories than animals housed in the NON housing (F (1, 43) = 16.437, $p < 0.05$) and males consumed more chip calories than females (F (1, 43) = 4.333, $p < 0.05$). A Tukey HSD comparison revealed that Super-Enriched males ate less cookies than Super-Enriched females, that ate less than Non-Enriched males, that ate less than Non-Enriched females (NON Males ≥ NON Females > SUP Males ≥ SUP Females). (See Table 26 and Figure 20d.)

Phase A2 – Standard Chow Only Period: GRAMS. Animals in the SUP housing ate significantly ate more than animals housed in the NON housing (F (1, 43) = 59.788, $p < 0.05$) and males ate more than females (F (1, 43) = 294.360, $p < 0.05$). A Tukey HSD comparison revealed that Super-Enriched males ate significantly less cookies than Super-Enriched females, that ate significantly less than Non-Enriched males, that ate significantly less than Non-Enriched females (NON Males > NON Females > SUP Males > SUP Females). (See Table 19j and Figure 22a.)

Phase A2 – Standard Chow Only Period: CALORIES. Animals in the SUP housing ate significantly more than animals housed in the NON housing (F (1, 43) = 59.788, $p < 0.05$) and males ate more than females (F (1, 43) = 294.360, $p < 0.05$). A Tukey HSD comparison revealed that Super-Enriched males ate significantly fewer calories than Super-Enriched females, that ate significantly fewer calories than Non-Enriched males, that ate significantly fewer

calories than Non-Enriched females (NON Males > NON Females > SUP Males > SUP Females). (See Table 19l and Figure 22b.)

Physical Activity (PA)

Home Cage Activity (HCA). HCA was measured 17 times during Experiment I (six times during Phase B and 11 times during Phase A2). The average number of animals moving, amount of activity, and level of activity were observed during each measurement. Each type of HCA is presented for each experimental phase.

Phase A1 – Standard Chow only Period. The Kruskal-Wallis Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity. Kruskal-Wallis mean rank orders indicated that all enrichment conditions were similar with regard to the mean number of animals moving: NON Females $\leq$ NON Males $\leq$ SUP Males $\leq$ SUP Females. Animals in the SUP conditions had the most activity, with Super-Enriched males ranked as the most active and Non-Enriched females as the least active: NON Females < NON Males < SUP Females < SUP Males. Super-Enriched males had the highest level of activity and Non-Enriched Females had the lowest rank for level of activity: NON Females < NON Males < SUP Females < SUP Males. (See Table 20a and Figures 23a - 23c.)

Phase B – Standard Chow with Sweet and Salty Foods Period. The Kruskal-Wallis Chi-Square was significant for number of animals moving, amount of activity, and level of activity. Kruskal-Wallis mean rank orders indicated that three conditions were similar with regard to the mean number of animals moving:

NON Females = SUP Males = SUP Females > NON Males. Animals in the SUP conditions had the most activity, with Super-Enriched Females ranked as the most active and Non-Enriched Females as the least active: NON Females < NON Males < SUP Males < SUP Females. Super-Enriched Females had the highest level of activity and Non-Enriched Males had the lowest rank for level of activity: NON Males < NON Females < SUP Males < SUP Females. (See Table 20b and Figures 23a - 23c.)

Phase A2 – Standard Chow Only Period. The Kruskal-Wallis Chi-Square was not significant for number of animals moving, but was significant for amount of activity, and level of activity. Kruskal-Wallis mean rank orders indicated that all enrichment conditions were similar with regard to the mean number of animals moving: NON Females ≤ NON Males ≤ SUP Males ≤ SUP Females. Animals in the SUP conditions had the most activity, with Super-Enriched females ranked as the most active and Non-Enriched females as the least active: NON Females < NON Males < SUP Males < SUP Females. Super-Enriched females had the highest level of activity and Non-Enriched Females had the lowest rank for level of activity: SUP Females < SUP Males < NON Males < NON Females. (See Table 20c and Figures 23a - 23c.)

Open Field (OF). A total of three OF trials examining horizontal and vertical activity were accomplished during Experiment III (1 per experimental phase - A1, B, and A2). Overall MANOVAs revealed that males and females exhibited similar total horizontal activity and vertical activity. Super-Enriched housed animals displayed significantly less total horizontal and vertical activity

than did the Non-Enriched housed animals. The results of the between-groups and the within-session analyses are presented separately for each Open-Field trial. Tables 21a - 21d present the details of the statistical analyses for the Open-Field trials. Figures 24a - 24c present graphical depictions of the horizontal and vertical activity for Open-Field trials. (Note: Males displayed significantly less vertical Activity during OF 3 which resulted in significant Sex X Housing interaction because OF 3 was the only time the difference was statistically significant. Despite this effect, OF activity levels between males and females were more similar than different.)

Phase A1 – Standard Chow Only Period, Open-Field Trial 1 Between Groups. Horizontal activity (F (1,43) = 242.947, $p < 0.05$) and vertical activity (F (1,43) = 118.804, $p < 0.05$) were significantly lower for animals housed in Super-Enrichment compared to animals housed in the Non-Enriched condition. There were no significant differences between males and females or Sex X Housing interactions. (See Table 21a & 21b and Figures 24a & 24b.)

Phase A1 – Standard Chow Only Period, Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity for vertical activity (F (11,473) = 78.060, $p < 0.05$) and vertical activity (F (11,473) = 58.025, $p < 0.05$), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity (F (22, 363) = 21.548, $p < 0.05$) and vertical activity (F (22, 363) = 10.430, $p < 0.05$) indicated that the decrease in activity over time was different for the three housing conditions such that the activity decreased most in

the Super-Enriched compared to the Non-Enriched housed animals. There was no Time X Sex interaction revealing that between the sexes there was no difference in the decreased activity over time. (See Table 21c & 21d and Figures 24c & 24d.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 2 Between Groups. Horizontal activity (F (1,43) = 97.225, p < 0.05) and vertical activity (F (F (1,43) = 45.398, p < 0.05) were significantly lower for animals in the SUP housing compared to animals in the NON housing. There was no difference in horizontal or vertical activity between males and females. There was no Sex X Housing interaction suggesting that activity was consistent between males and females and between housing conditions or that there was insufficient power to detect any interactions between Sex and Housing. (See Table 21a & 21b and Figures 24a & 24b.)

Phase B – Standard Chow with Sweet and Salty Foods Period, Open-Field Trial 2 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 473) = 65.474, p < 0.05) and vertical activity (F (11, 473) = 53.879, p < 0.05), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity (F (11, 473) = 8.318, p < 0.05) and vertical activity (F (11, 473) = 5.164, p < 0.05) indicated that over time, activity was greater in the NON housed animals compared to the SUP housed animals. (See Table 21c & 21d and Figures 24c & 24d.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 3 Between Groups. Horizontal activity (F (1,43) = 87.960, $p < 0.05$) and vertical activity (F (1,4 3) = 71.806, $p < 0.05$) were significantly lower for SUP compared to NON. Males moved more vertically than did females (F (1,43) = 4.083, $p < 0.05$). There was a Sex X Housing interaction for vertical activity (F (1,43) = 5.141, $p < 0.05$). (See Table 21a & 21b and Figures 24a & 24b.)

Phase A2 – Standard Chow Only Period, Open-Field Trial 3 Within Groups Within Session. Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 473) = 63.529, $p < 0.05$) and vertical activity (F (11, 363) = 37.862, $p < 0.05$), indicating that animal activity decreased over time in all conditions. In addition, a significant time by housing interaction for horizontal activity (F (22, 363) = 6.156, $p < 0.05$) and vertical activity (F (22, 363) = 2.396, $p < 0.05$) indicated that the decrease in activity over time was different between the three housing conditions such that the amount of activity decrease was greatest in the SUP compared to the NON housed animals. (See Table 21c & 21d and Figures 24c & 24d.)

Open-Field Results Summary. Animals in the Super-Enriched housing condition were less active during each of the three OF trials and these animals decreased activity more within each OF session compared with animals in the Non-Enriched housing conditions. These differences in activity in the housing conditions are consistent with the first two experiments in this series, and in other studies (Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996; Varty et al.,

2000; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). (See Figures 24a - 24d.)

Discussion: Experiment III

Experiment III was designed to replicate the findings from Experiments I & II, which examined the effects of environmental enrichment on: (1) body weight, when the different foods are available (2) food consumption, using bland foods and other, more preferred, foods, and (3) physical activity, including activity in an open field arena and within the home cage. In addition, Experiment III also directly compared these findings between male and female subjects.

The results of Experiment III indicated that animals housed in environmental enrichment had significantly lower body weights than animals not housed in enrichment. This finding is consistent with the first two experiments and with previous research (e.g., Sclafani & Springer, 1976; Tomchesson, 2004). Males had greater body weights than females. Animals housed in the Super-Enrichment had significantly lower BMI and LI scores compared to the Non-Enriched conditions. Males had significantly greater BMI scores than females. The lower indices suggest that animals housed in enriched environments had lower amounts of body fat (Maffie et al., 1995). There was no indication of poor health for the animals during Experiment III that could account for the lower BW, BMI, and LI.

Standard chow consumption was lower in the Super-enrichment condition. The decreased feeding again disappeared, as sweet and salty foods were made available. During this time, enrichment's effect to decrease body weight

appeared to be attenuated. With regard to the previous findings for specific foods during Phase B, many of the findings for different types of foods were not replicated. For example, males clearly preferred cookies to chips during Experiment I, whereas in Experiment III, males ate more chips than cookies. Also, the amount of standard chow was nearly identical for both males and females in the first two experiments, but in Experiment III Super-Enriched animals ate significantly less standard chow than the Non-Enriched housed animals for both males and females. Importantly, animals ate more of the preferred foods regardless of housing condition. Interestingly, the females ate more cookies than did the males (this finding was noted in the data from the first two experiments, but could not be directly compared). This finding is interesting because the males consumed more food in weight and calories for all foods except for cookies, which females consumed more than males.

Physical activity was measured in novel open field (OF) and home cages (HCA). OF activity (horizontal and vertical activity) was lower for the animals housed in enrichment. Males and females did not differ in the amount of their OF activity. Again, this decrease in OF activity is not consistent with a decrease in body weight but home cage activity results were consistent with lower body weights. For HCA, Super-Enriched housed animals moved more, had higher levels of activity, and weighed less than the animals in the Non-Enriched housing. Females had higher amounts and levels of activity compared to the males, which may help to explain why males seem to gain weight more rapidly than females.

In summary, Experiment III replicated several of the major findings from the first two experiments. Animals housed in environmental enrichment weighed less than animals in Non-Enriched housing, had lower BMI and LI scores, were more active in the OF environment, and were more active in their home cages. Food consumption was different for the specific types of foods; however, all animals ate more when more preferred foods were available, regardless of housing condition. In general, males and females appear to respond similarly to environmental enrichment.

SECTION III - ASSESSMENT & DISCUSSION

Assessment of Experimental Hypotheses

Hypotheses: Experiment I

Hypothesis 1 – EE, BW, BMI, and LI

Environmental enrichment will decrease body weight gains and body mass index such that: Super-Enriched < Enriched < Non-Enriched. ***Partially Confirmed -*** the rank order of the housing conditions by mean body weight, BMI, or LI were as predicted, however, only the Non-Enriched and Super-Enriched conditions was statistically different from each other for body weight. For LI and BMI, Super-Enriched and Enriched were not statistically different from each other, but were different from the Non-Enriched condition.

Hypothesis 2 – EE and FC

A) Environmental enrichment will decrease the amount of bland foods consumed compared to animals reared in Non-Enrichment such that: Super-Enriched < Enriched < Non-Enriched. ***Partially Confirmed -*** the rank order of

the housing conditions by the mean amount of food consumed was as predicted, however, Super-Enriched males ate significantly less than Enriched and Non-Enriched males. The difference between Enriched and Non-Enriched animals was not statistically different.

B) Animals raised in enriched and non-enriched housing conditions will eat similar amounts of cafeteria foods such that: Super-Enriched = Enriched = Non-Enriched. ***Confirmed***

Hypothesis 3 – EE and PA

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Super-Enriched < Enriched < Non-Enriched. ***Confirmed***

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Super-Enriched > Enriched > Non-Enriched. ***Confirmed***

Hypotheses: Experiment II

Hypothesis 1 – EE, BW, BMI, and LI

Environmental enrichment will decrease body weight gains and body mass index, and Lee index such that: Super-Enriched < Enriched < Non-Enriched. ***Partially Confirmed -*** the rank order of the housing conditions by mean body weight, BMI, or LI was as predicted, however, the Non-Enriched and Enriched conditions were not statistically different from each other, but were statistically different from the Super-Enriched housing condition.

Hypothesis 2 – EE and FC

A) Environmental enrichment will decrease the amount of bland foods consumed compared to animals reared in Non-Enrichment such that: Super-Enriched < Enriched < Non-Enriched. ***Confirmed***

B) Animals raised in enriched and non-enriched housing conditions will eat similar amounts of cafeteria foods such that: Super-Enriched = Enriched = Non-Enriched. ***Partially Confirmed -*** Super-Enriched females ate significantly fewer Oreos than Enriched females, that ate significantly fewer Oreo cookies than Non-Enriched females which caused a significant difference in the total food consumed. There were no differences in the amount of chips or standard foods consumed during Phase B.

Hypothesis 3 – EE and PA

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Super-Enriched < Enriched < Non-Enriched. ***Confirmed***

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Super-Enriched > Enriched > Non-Enriched. ***Confirmed***

Hypotheses: Experiment III

Hypothesis 1 – EE, BW, BMI and LI

Environmental enrichment will decrease body weight gains and body mass index such that: Super-Enriched < Non-Enriched. ***Confirmed***

Hypothesis 2 – EE and FC

A) The animals in the Super-Enrichment will consume less bland food than Non-Enriched housing. ***Confirmed***

B) Males will consume more standard rat chow than females. ***Confirmed***

C) Males and females will consume more food during Phase B compared to Phase A1 & A2 regardless of housing condition. ***Confirmed***

D) There will be a sex by housing interaction for the type of foods consumed during Phase B for females such that animals reared in the Super-Enriched condition will eat less than animals in the Non-Enriched condition during Phase B. However, there will be no significant differences in the amount of food consumed by males during Phase B. ***Not Confirmed -*** there were no Sex X Housing interactions for the amount of different types of foods consumed during Phase B.

Hypothesis 3 – EE and PA

A) Environmental enrichment will decrease the amount of physical activity in open-field trials compared to animals reared in Non-Enrichment such that: Super-Enriched < Non-Enriched. ***Confirmed***

B) Environmental enrichment will increase the amount of physical activity in the home cage compared to animals reared in Non-Enrichment such that: Super-Enriched > Non-Enriched. ***Confirmed***

C) Males and females will exhibit different activity levels in both Open-Field trials and Home Cage observations such that males will be less active in the Open-Field, but more active in the home cage. ***Not Confirmed -*** there were

no significant sex differences in OF activity levels and Super-Enriched females appeared to be the most active animals and Non-Enriched females appeared to be the overall least active animals.

General Discussion

Three separate experiments were conducted to examine the effects of environmental enrichment on body weight, feeding, and physical activity in male and female adolescent rats. This project highlights three key findings that have been well established in the research literature. First, rodent research models have proven to be a valuable means to conduct scientific research to inform the human condition in a variety of fields such as pharmacology, neuroscience, and psychology. For example, the common first line of experimental trials in pharmacological research is typically a rodent (mouse or rat) investigation. Ground-breaking research on psychological principles were conducted by such pioneers as Ivan Pavlov, B.F. Skinner, John Garcia, and Neal Miller. Animal models allow for more experimental control and more precise determination of causality. Interpretation and inference is used to extrapolate findings from animal experiments to humans. It is with this goal in mind that the present investigation was conducted and findings may be extrapolated to humans. The second major finding previously established in the research literature is that food consumption and physical activity are key factors that affect body weight (e.g. Premack & Premack, 1963; Sclafani & Springer, 1976; Sclafani & Gorman, 1977; Warwick, Synowski, & Bell, 2002; Lattanzio & Eikelboom, 2003). This particular project again confirms that energy intake, in the form of food consumption, and energy

expenditure, in the form of physical activity, are important factors in regulating body weight. The third previously established finding is that sweet and salty foods are preferred over bland foods. Research supporting this fact has been well established for many years as illustrated by the seminal research of Sclafani and Springer (1976).

This research project adds four major findings to the research literature. First, environmental enrichment is an important factor that can influence food consumption, physical activity levels, and body weight. Although other research has reported findings that may be consistent with environmental influences on these factors, to this investigator's knowledge, none has specifically investigated environmental enrichment's effects on all of these factors. The finding that environmental enrichment can decrease body weight through energy intake and energy expenditure is noteworthy. Providing an environment with both social and physical stimuli might increase the activity levels of the individual and decrease the consumption of bland foods, thereby controlling body.

The second important finding from this investigation is that environmental enrichment may not be a strong enough influence to offset food consumption of preferred foods. Understanding that more preferred foods may be a stronger reinforcer than having social and physical stimuli available in the environment is critical in our understanding of weight management. If available, preferred foods will be eaten, regardless of the availability for activity or engaging in activity. Also, the potency of the preferred foods offers another interesting finding. It is clear that despite housing condition, food consumption increased dramatically

when more preferred foods were available. In the group situations, the animals typically ate less preferred foods did than the isolated animals. The animals in both groups ate more than they did when standard bland chow alone was available.

The third major finding from this project is that male and female rats appear to respond similarly to environmental enrichment with regard to body weight, feeding, and physical activity. No other study has reported environmental enrichment's effects to decrease body weight, decrease feeding, decrease physical activity in an open field, and increase physical activity in the home cage in both adolescent male and female rats when compared to rats in a non-enriched housing condition. Although most of the major findings from Experiments I & II were replicated, there was some discrepancy in the details regarding the specific food types that were preferred by adolescent males and females. While the general replications suggest robust effects for enrichment's overall influence to decrease food consumption, further research is warranted before rendering any definitive conclusions regarding gender effects on the consumption of specific food types. Despite this inconsistency, there were no major gender differences in how adolescent male and female Sprague-Dawley rats' feeding, activity, and body weight were affected by environmental enrichment.

The fourth major finding from this project is the introduction of a new critical variable in environmental enrichment research, home cage activity. Previously, no other research reported on how environmental enrichment affects

animal activity in their home cages. In fact, the only activity reported in the literature was in a novel environment (i.e., Open-Field), which was not consistent with environmental enrichment's effect to decrease body weight. This finding is important for two main reasons. From a practical point of view, researchers need to be aware that providing social and physical stimuli into an animal's environment can introduce potential confounds for investigations, even for studies that are not examining environmental enrichment. It is commonplace for researchers to include environmental enrichment as a standard method to house animals during experiments regardless of their research question. Taken from Yerkes and Zoology, it is believed that enrichment is a more "humane" way of housing a captive animal. It is important to know that housing animals in larger groups or in bigger cages may affect body weight, daily physical activity, and feeding. Each of these factors potentially affects health, body composition, and metabolism, all of which are common dependent variables for a wide variety of investigations that have nothing to do with environmental enrichment. For example, an investigation examining a novel drug compound that houses animals in enrichment may lead to a different conclusion regarding body weight than would be reached if all animals were housed in isolation. The second reason home cage activity is an important introduction is that when extrapolating these findings to humans, it is important to provide more opportunities for social and physical stimuli in a child's environment to help manage their weight.

It is noteworthy that the Super-Enriched (SUP) condition used in this project probably is more similar to the natural environment in which rats live than

is the Non-Enriched (NON) condition. Therefore, the results of the present research may be regarded as the effects of “more natural” conditions to decrease body weight and increase home cage activity. However, the results may be regarded as the effects of “less natural” conditions to increase body weight and decrease home cage activity.

It also is important to note that the present research studied adolescent rats that became young adults over the course of the studies. Age affects body weight, food consumption, and physical activity. Therefore, the present findings apply to adolescent and young adult rats.

Potential Clinical Applications

There are several potential clinical applications of this project’s findings. The most basic application is eliminating access to unhealthy, great tasting, high caloric foods for adolescents and young adults attempting to control body weight. Many behavioral interventions (e.g., Thomas, 1995; Behavioral Choice Treatment, Sbrocco, et al., 1999, personal communication 2004; Treasure et al., 2003) suggest moderation and do not support the “complete elimination” of high caloric snacks. While moderation may be a more “acceptable” approach for adolescents and young adults concerned with weight loss, this project’s findings suggest that when high caloric, preferred foods are available, they are going to be eaten in excess. Therefore, such foods should not be available. Further, portion sizes should be monitored and controlled. Any excess food left over after the snack should be discarded because, according to the findings of this study, if the food is available it will be eaten. In line with this recommendation, snack

machines in military dorms and schools might limit the availability of unhealthy, high caloric, junk foods. An additional benefit of this intervention also may be the increase in physical activity (i.e., energy expenditure) required to “seek out” the high caloric foods. The practice of eliminating cues that may signal eating (e.g., junk food) is called stimulus control (i.e., using a tangible object or other cue as a reminder for a given behavior).

In many weight loss interventions, stimulus control is not simply getting rid of food, there are other ways to use stimulus control to limit food intake. For example, eating only at the dining room table, eating only at specific times of day, or not eating while watching TV. Given the findings from this study, providing the opportunities for activity becomes the stimulus control. This can be accomplished by providing bicycles to ride, stationary bikes in front of televisions or video games, posters of exercising, set times to exercise or engage in activity at home. Within the home environment, it may take great effort to provide opportunities to be more active. Providing balls or other exercise equipment, jump ropes, hula-hoops, or trampolines at home can encourage activity.

Ironically, findings from these investigations of animal behavior may be applied to cognitive interventions for weight management. As part of a multi-modal intervention for weight management, thought monitoring and challenging negative thoughts regarding one’s weight are standard interventions. It is generally accepted that people do not respond uniformly to therapeutic approaches. Consequently, adapting monothetic therapeutic interventions to each individual is typically recommended. As such, a thorough functional

analysis is important to guide the thought challenging and the specific thoughts to be challenged can be quite different from individual to individual. For example, the thought “I can’t lose weight” requires gathering evidence about one’s ability to lose weight. Conversely, the thought “I am too big to exercise” requires gathering evidence to support that “big people” can exercise, too. Each of these thoughts leads to different problem solving approaches. The results of this project suggest that interventions should also focus on the thoughts related to one’s ability to alter their environment (e.g., by including physical and social stimuli conducive to activity). In addition, thoughts regarding the ability to substitute more healthy foods into their daily schedule, go shopping, would also be important to include.

The most important clinical application is the provision of physical and social stimuli to promote activity in home, school, and work environments (wherever individuals spend the most time). Providing the opportunity to become more active is an important job for parents at home but also for the school systems because children spend a large portion of their day in school. Currently, many elementary, middle, and high schools have severely limited opportunities for physical activity and have inadequate physical education programs. Results of this study indicate that by providing a natural environment suited to physical activity and social stimulation (i.e., enrichment), children might be more likely to be more active, resulting in lower body weights. In addition to increasing the frequency, duration, and intensity of physical education classes, adding physical activity inside the classroom may be warranted. For example, incorporating

learning games requiring energy expenditure, such as standing and walking around the classroom, dancing in place, and limited exercise movements (e.g., stretching) might help increase activity.

Limitations and Future Directions

No research is perfect or without its limitations. Also, no research can examine every possible aspect of a given question. This project used only adolescent animals. The principal focus was on adolescents because of the current weight epidemic in children, but the findings of this study are limited to adolescents. Further investigation would be needed to determine whether the findings from this project generalize to additional age groups. This study used of only one strain of rat. The use of multiple strains could provide greater generalizability of findings across a more diverse population. The use of the Sprague-Dawley strain, the most widely used strain, allows for the greatest generalizability while using only one strain, but the use of multiple strains could provide greater utility to the findings should they replicate in the additional strains. Physical and social stimuli were used concurrently and not separated in this study. It is impossible to determine if a social or physical component is more important in enrichment's effects on body weight, feeding, and physical activity. For example, it is not clear if simply being around other animals led to an increase in home cage activity or if just having more room in the cage led to an increase in physical activity. This distinction could have important implications in how enrichment might be used to help manage an individual's body weight. This investigation did not examine metabolic activity. While it was beyond the scope

of this project, it is possible that changes that occur within the body in response to environmental enrichment cause alter metabolism. This project focused only on rats and did not use human subjects. In addition, the A-B-A design may have introduced time effects and order effects. The inclusion of B-A-B design or the use of a strictly between-subjects design would address these possibilities.

Several possible directions for future research could be explored. It would be valuable to examine the effect of environmental enrichment in different age groups and in multiple strains of rats to increase the generalizability of the findings. By separating social and physical components of environmental enrichment, important information may be obtained about how best to employ enrichment as a weight management strategy. More intriguing would be to conduct a human parallel study to examine a group of diverse individuals in a confined environment and see how they respond to environmental enrichment (e.g., as a member of the military, on a military installation during training, or during a remote assignment or deployment setting where the individuals are confined to a location for a given amount of time). It would be interesting to see how manipulating the environment to allow for increased or decreased social and physical stimulation impacts feeding, activity, and body weight.

It also would be valuable to examine possible explanations for the present results. For example, the SUP housing conditions provided increased floor space and opportunities for movement; increased numbers of social interactions; and probably altered brain morphology such as cortical thickness and increased dendritic branching (based on archived research). Future experiments should

separate and examine these possible mechanisms or mediators of the housing-induced effects on body weight, food consumption, and physical activity.

Summary and Conclusions

Providing opportunities for organisms to thrive and excel (i.e., environmental enrichment) has long-lasting positive biological and behavioral consequences such as increasing brain size, improving information processing and learning. In addition, enriched environments can affect physical activity and feeding, two factors that can increase or decrease body weight. Body weight is a concern because excessive body weight increases the risk for premature death and chronic illness. Three separate experiments were conducted to examine the effects of environmental enrichment on body weight, feeding, and physical activity in male and female adolescent rats.

From this research project it is clear that environmental conditions, particularly housing conditions, are important in regulating body weight for adolescent male and female Sprague-Dawley rats. Body weight is significantly reduced, the consumption of bland foods is reduced, and providing physical and social stimuli in the home cage environment increases activity in that cage. The decrease in body weight may be accomplished through a decreased feeding that occurs when bland foods are available or by an increase in daily activity that seems to occur in the enriched environments. This project provides evidence for the importance for considering animals' home cage activity and the use of environmental enrichment in animal research. These findings also provide evidence for some of the clinical interventions already used in managing weight

loss or gain (e.g., stimulus control, social support). Additionally, these findings suggest that increasing opportunities for social and physical interactions within the home environment is a potential means to improve weight management interventions for adolescent and young adult humans.

SECTION IV – TABLES, FIGURES, and REFERENCES

Tables

Table 1. Experiment I Timeline

Date	Day of Study	Measure/Activity
17 Aug	Day 1 - **Phase 1**	New Housing – FC & BW
18Aug	Day 2	HCA
19 Aug	Day 3	FC & OF
20 Aug	Day 4	
21 Aug	Day 5	FC & BW
22 Aug	Day 6	
23 Aug	Day 7	HCA & FC
24 Aug	Day 8	
25Aug	Day 9	FC
26 Aug	Day 10	BW
27 Aug	Day 11	FC
28 Aug	Day 12	
29 Aug	Day 13	FC & OF
30 Aug	Day 14	HCA & BW
31 Aug	Day 15 **- Phase 2** Day 1	FC
1 Sep	Day 17 Day 2	HCA
2 Sep	Day 18 Day 3	FC & OF
3 Sep	Day 19 Day 4	
4 Sep	Day 20 Day 5	FC & BW
5 Sep	Day 21 Day 6	
6 Sep	Day 22 Day 7	HCA
7 Sep	Day 23 Day 8	
8 Sep	Day 24 Day 9	FC
9 Sep	Day 25 Day 10	BW
10 Sep	Day 26 Day 11	FC
11 Sep	Day 27 Day 12	
12 Sep	Day 28 Day 13	FC & OF
13 Sep	Day 29 Day 14	HCA & BW
14 Sep	Day 30 **- Phase 3** Day 1	FC
15 Sep	Day 31 Day 2	HCA
16 Sep	Day 32 Day 3	FC & OF
17 Sep	Day 33 Day 4	
18 Sep	Day 34 Day 5	FC & BW
19 Sep	Day 35 Day 6	
20 Sep	Day 36 Day 7	HCA & FC
21 Sep	Day 37 Day 8	
22 Sep	Day 38 Day 9	FC
23 Sep	Day 39 Day 10	BW
24 Sep	Day 40 Day 11	FC
25 Sep	Day 41 Day 12	
26 Sep	Day 41 Day 13	FC & OF
27 Sep	Day 43 Day 14	HCA & BW
28 Sep	Day 44 Day 15	FC
29Sep	Day 45 Day 16	HCA
30 Sep	Day 46 Day 17	FC
1 Oct	Day 47 Day 18	
2 Oct	Day 48 Day 19	FC & BW
3 Oct	Day 49 Day 20	HCA
4 Oct	Day 50 Day 21	Sacrifice

BW = Body Weight Measurement
FC = Food Consumption
HCA = Home Cage Activity
OF = Open Field (locomotor)

Table 2. Experiment I Body Weight

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	13806.189	10	1380.619	8.185	.000	.204	1.000
Time * Housing	15522.627	20	776.131	4.601	.000	.223	1.000
Error(time)	53975.995	320	168.675				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	23472.350	2	11736.175	3.474	.043	.178	.608
Error	108105.725	32	3378.304				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
					95% Confidence Interval	
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.(a)	Lower Bound	Upper Bound
Non-Enriched	Enriched	10.920	7.606	.482	-8.296	30.136
	Super-Enriched	19.204(*)	7.287	.039	.793	37.616
Enriched	Non-Enriched	-10.920	7.606	.482	-30.136	8.296
	Super-Enriched	8.284	7.254	.786	-10.042	26.611
Super-Enriched	Non-Enriched	-19.204(*)	7.287	.039	-37.616	-.793
	Enriched	-8.284	7.254	.786	-26.611	10.042

Table 3. Experiment I Body Mass Index

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	529.719	2	264.859	6.682	.004	.288	.888
Error	1308.032	33	39.637				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	7.87103(*)	2.570257	.012	1.56415	14.17792
	Super-Enriched	8.37961(*)	2.570257	.007	2.07273	14.68649
Enriched	Non-Enriched	-7.87103(*)	2.570257	.012	-14.17792	-1.56415
	Super-Enriched	.50857	2.570257	.979	-5.79831	6.81546
Super-Enriched	Non-Enriched	-8.37961(*)	2.570257	.007	-14.68649	-2.07273
	Enriched	-.50857	2.570257	.979	-6.81546	5.79831

Table 4. Experiment I Lee Index

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	1274.370	2	637.185	4.919	.013	.230	.769
Error	4274.330	33	129.525				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	13.19140(*)	4.646238	.020	1.79049	24.59232
	Super-Enriched	11.96100(*)	4.646238	.038	.56009	23.36191
Enriched	Non-Enriched	-13.19140(*)	4.646238	.020	-24.59232	-1.79049
	Super-Enriched	-1.23040	4.646238	.962	-12.63131	10.17051
Super-Enriched	Non-Enriched	-11.96100(*)	4.646238	.038	-23.36191	-.56009
	Enriched	1.23040	4.646238	.962	-10.17051	12.63131

Table 5a. Experiment I Phase A1 Gram Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	29.129	2	14.564	3.835	.032	.189	.655
Error	125.335	33	3.798				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	.007	.7956	1.000	-1.945	1.959
	Super-Enriched	1.912	.7956	.056	-.041	3.864
Enriched	Non-Enriched	-.007	.7956	1.000	-1.959	1.945
	Super-Enriched	1.905	.7956	.057	-.048	3.857
Super-Enriched	Non-Enriched	-1.912	.7956	.056	-3.864	.041
	Enriched	-1.905	.7956	.057	-3.857	.048

Table 5b. Experiment I Phase A1 Calorie Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	3.172	2	1.586	3.835	.032	.189	.655
Error	13.649	33	.414				

Post Hoc Comparisons - Tukey HSD						
(I) housing	(J) housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	.002	.2626	1.000	-.642	.647
	Super-Enriched	.631	.2626	.056	-.013	1.275
Enriched	Non-Enriched	-.002	.2626	1.000	-.647	.642
	Super-Enriched	.629	.2626	.057	-.016	1.273
Super-Enriched	Non-Enriched	-.631	.2626	.056	-1.275	.013
	Enriched	-.629	.2626	.057	-1.273	.016

Table 5c. Experiment I Phase B Total Gram Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	7.400	2	3.700	1.151	.329	.065	.235
Error	106.056	33	3.214				

Table 5d. Experiment I Phase B Total Calories Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	158.345	2	79.173	2.923	.068	.150	.532
Error	893.873	33	27.087				

Table 5e. Experiment I Phase B Standard Chow Grams Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	23.053	2	11.527	3.767	.034	.186	.647
Error	100.990	33	3.060				

Post Hoc Comparisons - Tukey HSD						
(I) housing	(J) housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	-1.5447	.71418	.093	-3.2971	.2077
	Super-Enriched	-1.8174(*)	.71418	.041	-3.5698	-.0649
Enriched	Non-Enriched	1.5447	.71418	.093	-.2077	3.2971
	Super-Enriched	-.2727	.71418	.923	-2.0251	1.4798
Super-Enriched	Non-Enriched	1.8174(*)	.71418	.041	.0649	3.5698
	Enriched	.2727	.71418	.923	-1.4798	2.0251

Table 5f. Experiment I Phase B Standard Chow Calories Consumption

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	10.042	2	5.021	3.767	.034	.186	.647
Error	43.991	33	1.333				

Post Hoc Comparisons - Tukey HSD

(I) housing	(J) housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	-1.5447	.71418	.093	-3.2971	.2077
	Super-Enriched	-1.8174(*)	.71418	.041	-3.5698	-.0649
Enriched	Non-Enriched	1.5447	.71418	.093	-.2077	3.2971
	Super-Enriched	-.2727	.71418	.923	-2.0251	1.4798
Super-Enriched	Non-Enriched	1.8174(*)	.71418	.041	.0649	3.5698
	Enriched	.2727	.71418	.923	-1.4798	2.0251

Table 5g. Experiment I Phase B Oreo Cookie Grams Consumption

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	1.051	2	.526	.343	.712	.020	.100
Error	50.637	33	1.534				

Table 5h. Experiment I Phase B Oreo Cookie Calories

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	23.319	2	11.660	.343	.712	.020	.100
Error	1123.335	33	34.040				

Table 5i. Experiment I Phase B Lay’s Chip Gram Consumption

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	3.831	2	1.915	2.078	.141	.112	.397
Error	30.418	33	.922				

Table 5j. Experiment I Phase B Lay's Chip Calorie Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	110.061	2	55.030	2.078	.141	.112	.397
Error	873.909	33	26.482				

Table 5k. Experiment I Phase A2 Standard Chow Gram Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	65.730	2	32.865	1.654	.207	.091	.323
Error	125.324	33	3.798				

Table 5l. Experiment I Phase A2 Standard Chow Calorie Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	5.528	2	2.764	1.532	.231	.085	.302
Error	59.552	33	1.805				

Table 6a. Experiment I Phase B Home Cage Activity

Kruskal-Wallis Test	**Housing Condition**	**N**	**Mean Rank**
Number of Animals Moving	Non-Enriched	6	6.08
	Enriched	6	9.75
	Super-Enriched	6	12.67
	Total	18	
Amount of Activity	Non-Enriched	6	5.00
	Enriched	6	10.08
	Super-Enriched	6	13.42
	Total	18	
Level of Activity	Non-Enriched	6	4.67
	Enriched	6	11.92
	Super-Enriched	6	11.92
	Total	18	
Grouping Variable: Housing Condition	**Number Moving**	**Activity Amount**	**Activity Level**
Chi-Square	4.769	8.493	7.517
Df	2	2	2
Asymp. Sig.	.092	.014	.023

Table 6b. Experiment I Phase A2 Home Cage Activity

Kruskal-Wallis Test	Housing Condition	N	Mean Rank
Number of Animals Moving	Non-Enriched	11	10.59
	Enriched	11	21.45
	Super-Enriched	11	18.95
	Total	33	
Amount of Activity	Non-Enriched	11	8.50
	Enriched	11	20.91
	Super-Enriched	11	21.59
	Total	33	
Level of Activity	Non-Enriched	11	7.82
	Enriched	11	20.00
	Super-Enriched	11	23.18
	Total	33	
Grouping Variable: Housing Condition	**Animals Moving**	**Amount Activity**	**Level Activity**
Chi-Square	10.293	13.233	16.106
Df	2	2	2
Asymp. Sig.	.006	.001	.000

Table 7a. Experiment I Open Field MANOVA

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TOTHAC1	Contrast	100342713.722	2	50171356.861	7.716	.002	.319	.929
	Error	214583221.917	33	6502521.876				
TOTVAC1	Contrast	276447.167	2	138223.583	3.362	.047	.169	.594
	Error	1356918.833	33	41118.753				
TOTHAC2	Contrast	471468284.056	2	235734142.028	20.947	.000	.559	1.000
	Error	371374981.833	33	11253787.328				
TOTVAC2	Contrast	1332567.167	2	666283.583	14.120	.000	.461	.997
	Error	1557191.833	33	47187.631				
TOTHAC3	Contrast	289371641.167	2	144685820.583	13.394	.000	.448	.996
	Error	356477343.833	33	10802343.753				
TOTVAC3	Contrast	643658.167	2	321829.083	8.966	.001	.352	.960
	Error	1184473.833	33	35893.146				
TOTHAC4	Contrast	319840198.389	2	159920099.194	9.464	.001	.365	.969
	Error	557616582.833	33	16897472.207				
TOTVAC4	Contrast	1380348.667	2	690174.333	6.198	.005	.273	.862
	Error	3674640.083	33	111352.730				
TOTHAC5	Contrast	404176753.500	2	202088376.750	24.785	.000	.600	1.000
	Error	269072695.250	33	8153718.038				
TOTVAC5	Contrast	2599014.500	2	1299507.250	13.373	.000	.448	.996
	Error	3206798.250	33	97175.705				
TOTHAC6	Contrast	632487582.167	2	316243791.083	16.079	.000	.494	.999
	Error	649038160.833	33	19667823.056				
TOTVAC6	Contrast	8956486.167	2	4478243.083	11.735	.000	.416	.990
	Error	12593082.583	33	381608.563				

Table 7b. Experiment I OF 1 Horizontal Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	49633230.414	11	4512111.856	30.459	.000	.480	1.000
TIME *HOUSING	12470439.079	22	566838.140	3.826	.000	.188	1.000
Error(TIME)	53773780.924	363	148137.138				

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	8361892.810	2	4180946.405	7.716	.002	.319	.929
Error	17881935.160	33	541876.823				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	83.042	86.753	1.000	-135.768	301.851
	Super-Enriched	327.757(*)	86.753	.002	108.948	546.566
Enriched	Non-Enriched	-83.042	86.753	1.000	-301.851	135.768
	Super-Enriched	244.715(*)	86.753	.024	25.906	463.525
Super-Enriched	Non-Enriched	-327.757(*)	86.753	.002	-546.566	-108.948
	Enriched	-244.715(*)	86.753	.024	-463.525	-25.906

Table 7c. Experiment I OF1 Vertical Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	176856.944	11	16077.904	14.704	.000	.308	1.000
TIME * HOUSING	60230.292	22	2737.741	2.504	.000	.132	.999
Error(TIME)	396919.597	363	1093.442				

Tests of Between-Subjects Effects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	23037.264	2	11518.632	3.362	.047	.169	.594
Error	113076.569	33	3426.563				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	.243	6.899	1.000	-17.157	17.643
	Super-Enriched	15.611	6.899	.091	-1.789	33.011
Enriched	Non-Enriched	-.243	6.899	1.000	-17.643	17.157
	Super-Enriched	15.368	6.899	.099	-2.032	32.768
Super-Enriched	Non-Enriched	-15.611	6.899	.091	-33.011	1.789
	Enriched	-15.368	6.899	.099	-32.768	2.032

Table 7d. Experiment I OF 2 Horizontal Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	105817108.935	11	9619737.176	47.499	.000	.590	1.000
TIME*HOUSING	28284114.384	22	1285641.563	6.348	.000	.278	1.000
Error(TIME)	73515888.514	363	202523.109				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	39289023.671	2	19644511.836	20.947	.000	.559	1.000
Error	30947915.153	33	937815.611				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	130.563	114.128	.783	-157.293	418.418
	Super-Enriched	694.944(*)	114.128	.000	407.089	982.800
Enriched	Non-Enriched	-130.563	114.128	.783	-418.418	157.293
	Super-Enriched	564.382(*)	114.128	.000	276.527	852.237
Super-Enriched	Non-Enriched	-694.944(*)	114.128	.000	-982.800	-407.089
	Enriched	-564.382(*)	114.128	.000	-852.237	-276.527

Table 7e. Experiment I OF 2 Vertical Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	595294.750	11	54117.705	39.802	.000	.547	1.000
TIME*HOUSING	150607.569	22	6845.799	5.035	.000	.234	1.000
Error(TIME)	493566.347	363	1359.687				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	111047.264	2	55523.632	14.120	.000	.461	.997
Error	129765.986	33	3932.303				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	-6.056	7.390	1.000	-24.695	12.584
	Super-Enriched	30.576(*)	7.390	.001	11.937	49.216
Enriched	Non-Enriched	6.056	7.390	1.000	-12.584	24.695
	Super-Enriched	36.632(*)	7.390	.000	17.992	55.272
Super-Enriched	Non-Enriched	-30.576(*)	7.390	.001	-49.216	-11.937
	Enriched	-36.632(*)	7.390	.000	-55.272	-17.992

Table 7f. Experiment I OF 3 Horizontal Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	115655171.583	11	10514106.508	47.521	.000	.590	1.000
TIME*HOUSING	21794337.069	22	990651.685	4.477	.000	.213	1.000
Error(TIME)	80314194.514	363	221251.225				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	24114303.431	2	12057151.715	13.394	.000	.448	.996
Error	29706445.319	33	900195.313				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	1.528	111.816	1.000	-280.495	283.550
	Super-Enriched	501.951(*)	111.816	.000	219.929	783.974
Enriched	Non-Enriched	-1.528	111.816	1.000	-283.550	280.495
	Super-Enriched	500.424(*)	111.816	.000	218.401	782.446
Super-Enriched	Non-Enriched	-501.951(*)	111.816	.000	-783.974	-219.929
	Enriched	-500.424(*)	111.816	.000	-782.446	-218.401

Table 7g. Experiment I OF3 Vertical Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	576140.056	11	52376.369	40.548	.000	.551	1.000
TIME*HOUSING	68415.764	22	3109.807	2.408	.000	.127	.998
Error(TIME)	468887.514	363	1291.701				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	53638.181	2	26819.090	8.966	.001	.352	.960
Error	98706.153	33	2991.096				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	1.660	6.445	1.000	-14.597	17.916
	Super-Enriched	24.424(*)	6.445	.002	8.167	40.680
Enriched	Non-Enriched	-1.660	6.445	1.000	-17.916	14.597
	Super-Enriched	22.764(*)	6.445	.004	6.507	39.021
Super-Enriched	Non-Enriched	-24.424(*)	6.445	.002	-40.680	-8.167
	Enriched	-22.764(*)	6.445	.004	-39.021	-6.507

Table 7h. Experiment I OF 4 Horizontal Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	194173584.444	11	17652144.040	85.290	.000	.721	1.000
TIME*HOUSING	20375592.681	22	926163.304	4.475	.000	.213	1.000
Error(TIME)	75128572.542	363	206965.765				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	26335164.542	2	13167582.271	9.604	.001	.368	.971
Error	45243536.458	33	1371016.256				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	381.562(*)	137.992	.028	33.516	729.609
	Super-Enriched	597.146(*)	137.992	.000	249.100	945.192
Enriched	Non-Enriched	-381.562(*)	137.992	.028	-729.609	-33.516
	Super-Enriched	215.583	137.992	.383	-132.463	563.629
Super-Enriched	Non-Enriched	-597.146(*)	137.992	.000	-945.192	-249.100
	Enriched	-215.583	137.992	.383	-563.629	132.463

Table 7i. Experiment I OF 4 Vertical Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	1309952.303	11	119086.573	53.655	.000	.619	1.000
TIME*HOUSING	131486.981	22	5976.681	2.693	.000	.140	.999
Error(TIME)	805679.299	363	2219.502				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	112342.019	2	56171.009	6.186	.005	.273	.862
Error	299639.451	33	9079.983				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	28.375(*)	11.230	.049	.051	56.699
	Super-Enriched	37.986(*)	11.230	.006	9.662	66.310
Enriched	Non-Enriched	-28.375(*)	11.230	.049	-56.699	-.051
	Super-Enriched	9.611	11.230	1.000	-18.713	37.935
Super-Enriched	Non-Enriched	-37.986(*)	11.230	.006	-66.310	-9.662
	Enriched	-9.611	11.230	1.000	-37.935	18.713

Table 7j. Experiment I OF 5 Horizontal Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	181861445.674	11	16532858.698	80.477	.000	.709	1.000
TIME*HOUSING	13818534.431	22	628115.201	3.057	.000	.156	1.000
Error(TIME)	74573405.479	363	205436.379				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	33681396.125	2	16840698.063	24.785	.000	.600	1.000
Error	22422724.604	33	679476.503				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	275.146(*)	97.145	.023	30.125	520.167
	Super-Enriched	679.854(*)	97.145	.000	434.833	924.875
Enriched	Non-Enriched	-275.146(*)	97.145	.023	-520.167	-30.125
	Super-Enriched	404.708(*)	97.145	.001	159.688	649.729
Super-Enriched	Non-Enriched	-679.854(*)	97.145	.000	-924.875	-434.833
	Enriched	-404.708(*)	97.145	.001	-649.729	-159.688

Table 7k. Experiment I OF 5 Vertical Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	1509106.562	11	137191.506	61.300	.000	.650	1.000
TIME*HOUSING	136050.625	22	6184.119	2.763	.000	.143	1.000
Error(TIME)	812405.896	363	2238.033				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	216584.542	2	108292.271	13.373	.000	.448	.996
Error	267233.188	33	8097.975				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	22.646	10.605	.121	-4.103	49.395
	Super-Enriched	54.583(*)	10.605	.000	27.835	81.332
Enriched	Non-Enriched	-22.646	10.605	.121	-49.395	4.103
	Super-Enriched	31.938(*)	10.605	.015	5.189	58.686
Super-Enriched	Non-Enriched	-54.583(*)	10.605	.000	-81.332	-27.835
	Enriched	-31.938(*)	10.605	.015	-58.686	-5.189

Table 7l. Experiment I OF 6 Horizontal Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	178523926.639	11	16229447.876	72.093	.000	.686	1.000
TIME*HOUSING	19018308.764	22	864468.580	3.840	.000	.189	1.000
Error(TIME)	81718428.597	363	225119.638				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	52707298.514	2	26353649.257	16.079	.000	.494	.999
Error	54086513.403	33	1638985.255				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference(a)	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	261.132	150.876	.279	-119.410	641.674
	Super-Enriched	836.181(*)	150.876	.000	455.638	1216.723
Enriched	Non-Enriched	-261.132	150.876	.279	-641.674	119.410
	Super-Enriched	575.049(*)	150.876	.002	194.506	955.591
Super-Enriched	Non-Enriched	-836.181(*)	150.876	.000	-1216.723	-455.638
	Enriched	-575.049(*)	150.876	.002	-955.591	-194.506

Table 7m. Experiment I OF 6 Vertical Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	2005099.618	11	182281.783	41.351	.000	.556	1.000
TIME*HOUSING	216221.597	22	9828.254	2.230	.001	.119	.996
Error(TIME)	1600161.368	363	4408.158				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HOUSING	746373.847	2	373186.924	11.735	.000	.416	.990
Error	1049423.549	33	31800.714				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) HOUSING	(J) HOUSING	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	36.660	21.016	.271	-16.347	89.667
	Super-Enriched	100.590(*)	21.016	.000	47.583	153.597
Enriched	Non-Enriched	-36.660	21.016	.271	-89.667	16.347
	Super-Enriched	63.931(*)	21.016	.014	10.923	116.938
Super-Enriched	Non-Enriched	-100.590(*)	21.016	.000	-153.597	-47.583
	Enriched	-63.931(*)	21.016	.014	-116.938	-10.923

Table 8. Experiment II Timeline

Date	Day of Study		Measure/Activity
25 Jan	Day 1 - **Phase 1**		New Housing – FC & BW
26 Jan	Day 2		HCA
27 Jan	Day 3		FC & OF
28 Jan	Day 4		
29 Jan	Day 5		FC & BW
30 Jan	Day 6		
31 Jan	Day 7		HCA & FC
1 Feb	Day 8		
2 Feb	Day 9		FC
3 Feb	Day 10		BW
4 Feb	Day 11		FC
5 Feb	Day 12		
6 Feb	Day 13		FC
7 Feb	Day 14		HCA & BW& OF
8 Feb	Day 15 **- Phase 2**	Day 1	FC
9 Feb	Day 17	Day 2	HCA
10 Feb	Day 18	Day 3	FC & OF
11 Feb	Day 19	Day 4	
12 Feb	Day 20	Day 5	FC & BW
13 Feb	Day 21	Day 6	
14 Feb	Day 22	Day 7	HCA
15 Feb	Day 23	Day 8	
16 Feb	Day 24	Day 9	FC
17 Feb	Day 25	Day 10	BW
18 Feb	Day 26	Day 11	FC
19 Feb	Day 27	Day 12	
20 Feb	Day 28	Day 13	FC
21 Feb	Day 29	Day 14	HCA & BW& OF
22 Feb	Day 30 **- Phase 3**	Day 1	FC
23 Feb	Day 31	Day 2	HCA
24 Feb	Day 32	Day 3	FC & OF
25 Feb	Day 33	Day 4	
26 Feb	Day 34	Day 5	FC & BW
27 Feb	Day 35	Day 6	
28 Feb	Day 36	Day 7	HCA & FC
29 Feb	Day 37	Day 8	
30 Feb	Day 38	Day 9	FC
31 Feb	Day 39	Day 10	BW
1 Mar	Day 40	Day 11	FC
2 Mar	Day 41	Day 12	
3 Mar	Day 41	Day 13	FC
4 Mar	Day 43	Day 14	HCA & BW
5 Mar	Day 44	Day 15	FC
6 Mar	Day 45	Day 16	HCA
7 Mar	Day 46	Day 17	FC
8 Mar	Day 47	Day 18	
9 Mar	Day 48	Day 19	FC & BW
10 Mar	Day 49	Day 20	HCA & OF
11 Mar	Day 50	Day 21	Sacrifice

BW = Body Weight Measurement
FC = Food Consumption
HCA = Home Cage Activity
OF = Open Field (locomotor)

Table 9. Experiment II Body Weight

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	9486.911	10	948.691	12.554	.000	.282	1.000
Time * Housing	4658.486	20	232.924	3.082	.000	.162	1.000
Error(time)	24182.357	320	75.570				

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BW Base	21314.159	1	21314.159	12.380	.001	.279	.927
Housing	26951.043	2	13475.522	7.827	.002	.328	.932
Error	55094.273	32	1721.696				

Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	4.687	5.130	1.000	-8.274	17.649
	Super-Enriched	19.440(*)	5.115	.002	6.518	32.362
Enriched	Non-Enriched	-4.687	5.130	1.000	-17.649	8.274
	Super-Enriched	14.753(*)	5.163	.022	1.709	27.797
Super-Enriched	Non-Enriched	-19.440(*)	5.115	.002	-32.362	-6.518
	Enriched	-14.753(*)	5.163	.022	-27.797	-1.709

Table 10. Experiment II Body Mass Index

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	263.824	2	131.912	6.836	.003	.293	.895
Error	636.769	33	19.296				

Post Hoc Comparisons - Tukey HSD

(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	-.148	1.793	1.000	-4.671	4.375
	Super-Enriched	5.667(*)	1.793	.010	1.144	10.190
Enriched	Non-Enriched	.148	1.793	1.000	-4.375	4.671
	Super-Enriched	5.815(*)	1.793	.008	1.292	10.338
Super-Enriched	Non-Enriched	-5.667(*)	1.793	.010	-10.190	-1.144
	Enriched	-5.815(*)	1.793	.008	-10.338	-1.292

Table 11. Experiment II Lee Index

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	782.285	2	391.142	4.342	.021	.208	.713
Error	2972.896	33	90.088				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	-2.529	3.875	1.000	-12.302	7.244
	Super-Enriched	8.379	3.875	.114	-1.395	18.152
Enriched	Non-Enriched	2.529	3.875	1.000	-7.244	12.302
	Super-Enriched	10.908(*)	3.875	.024	1.134	20.681
Super-Enriched	Non-Enriched	-8.379	3.875	.114	-18.152	1.395
	Enriched	-10.908(*)	3.875	.024	-20.681	-1.134

Table 12a. Experiment II Phase A1 Gram Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	26.820	2	13.410	2.526	.095	.133	.470
Error	175.161	33	5.308				

Table 12b. Experiment II Phase A1 Calorie Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
Housing	9.890	2	4.945	18.766	.000	.532	1.000
Error	8.696	33	.264				

Tests of Between-Subjects Effects Post Hoc Comparisons - Tukey HSD						
(I) housing	(J) housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	1.237 (*)	.2095671	.000	.723030	1.751499
	Super-Enriched	.9155 (*)	.2095671	.000	.401280	1.429749
Enriched	Non-Enriched	-1.237 (*)	.2095671	.000	-1.751499	-.723030
	Super-Enriched	-.3217	.2095671	.288	-.835985	.192485
Super-Enriched	Non-Enriched	-.9155 (*)	.2095671	.000	-1.429749	-.401280
	Enriched	.3217	.2095671	.288	-.192485	.835985

Table 12c. Experiment II Phase B Total Gram Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	69.627	2	34.814	6.017	.006	.267	.852
Error	190.932	33	5.786				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	1.273333	.9819893	.407	-1.136267	3.682933
	Super-Enriched	3.372976(*)	.9819893	.004	.963376	5.782576
Enriched	Non-Enriched	-1.273333	.9819893	.407	-3.682933	1.136267
	Super-Enriched	2.099643	.9819893	.098	-.309957	4.509243
Super-Enriched	Non-Enriched	-3.372976(*)	.9819893	.004	-5.782576	-.963376
	Enriched	-2.099643	.9819893	.098	-4.509243	.309957

Table 12d. Experiment II Phase B Total Calories Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	2323.958	2	1161.979	12.753	.000	.436	.994
Error	3006.718	33	91.113				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	10.709573(*)	3.8968507	.025	1.147502	20.271643
	Super-Enriched	19.654212(*)	3.8968507	.000	10.092141	29.216283
Enriched	Non-Enriched	-10.709573(*)	3.8968507	.025	-20.271643	-1.147502
	Super-Enriched	8.944639	3.8968507	.070	-.617432	18.506710
Super-Enriched	Non-Enriched	-19.654212(*)	3.8968507	.000	-29.216283	-10.092141
	Enriched	-8.944639	3.8968507	.070	-18.506710	.617432

Table 12e. Experiment II Phase B Standard Chow Grams Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	26.820	2	13.410	2.526	.095	.133	.470
Error	175.161	33	5.308				

Table 12f. Experiment II Phase B Standard Chow Calories Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	3.479	2	1.739	2.526	.095	.133	.470
Error	22.722	33	.689				

Table 12g. Experiment II Phase B Oreo Cookie Grams Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	2007.224	2	1003.612	13.889	.000	.457	.997
Error	2384.527	33	72.258				

Post Hoc Comparisons -Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	8.9804(*)	3.470	.037	.465	17.495
	Super-Enriched	18.2894(*)	3.470	.000	9.773	26.804
Enriched	Non-Enriched	-8.9804(*)	3.470	.037	-17.495	-.465
	Super-Enriched	9.3090(*)	3.470	.030	.793	17.824
Super-Enriched	Non-Enriched	-18.2894(*)	3.470	.000	-26.804	-9.773
	Enriched	-9.3090(*)	3.470	.030	-17.824	-.793

Table 12h. Experiment II Phase B Oreo Cookie Calories Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	2007.224	2	1003.612	13.889	.000	.457	.997
Error	2384.527	33	72.258				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	8.980 (*)	3.470	.037	.464	17.495
	Super-Enriched	18.289 (*)	3.470	.000	9.773	26.804
Enriched	Non-Enriched	-8.980 (*)	3.470	.037	-17.495	-.464
	Super-Enriched	9.308 (*)	3.470	.030	.793	17.824
Super-Enriched	Non-Enriched	-18.289 (*)	3.470	.000	-26.804	-9.773
	Enriched	-9.308 (*)	3.470	.030	-17.824	-.793

Table 12i. Experiment II Phase B Lay's Chip Grams Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	5.580	2	2.790	.666	.521	.039	.152
Error	138.340	33	4.192				

Table 12j. Experiment II Phase B Lay's Chip Calorie Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	40.079	2	20.039	.666	.521	.039	.152
Error	993.610	33	30.109				

Table 12k. Experiment II Phase A2 Standard Chow Gram Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	83.267	2	41.633	8.749	.001	.347	.956
Error	157.026	33	4.758				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	2.019832	.890	.075	-.165370	4.205034
	Super-Enriched	-1.700901	.890	.152	-3.886103	.484301
Enriched	Non-Enriched	-2.019832	.890	.075	-4.205034	.165370
	Super-Enriched	-3.720733(*)	.890	.001	-5.905935	-1.535531
Super-Enriched	Non-Enriched	1.700901	.890	.152	-.484301	3.886103
	Enriched	3.720733(*)	.890	.001	1.535531	5.905935

Table 12l. Experiment I Phase A2 Standard Chow Calorie Consumption

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	2323.958	2	1161.979	12.753	.000	.436	.994
Error	3006.718	33	91.113				

Post Hoc Comparisons - Tukey HSD						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	10.709(*)	3.896	.025	1.147502	20.271643
	Super-Enriched	19.654(*)	3.896	.000	10.092141	29.216283
Enriched	Non-Enriched	-10.709(*)	3.896	.025	-20.271643	-1.147502
	Super-Enriched	8.944	3.896	.070	-.617432	18.506710
Super-Enriched	Non-Enriched	-19.654(*)	3.896	.000	-29.216283	-10.092141
	Enriched	-8.944	3.896	.070	-18.506710	.617432

Table 13a. Experiment II Phase A1 Home Cage Activity Kruskal Wallis Test

Kruskal-Wallis Test	Housing Condition	N	Mean Rank
Number of Animals Moving	Non-Enriched	10	13.10
	Enriched	10	14.70
	Super-Enriched	10	18.70
	Total	30	
Amount of Activity	Non-Enriched	10	10.70
	Enriched	10	14.75
	Super-Enriched	10	21.05
	Total	30	
Level of Activity	Non-Enriched	10	10.50
	Enriched	10	13.90
	Super-Enriched	10	22.10
	Total	30	
Grouping Variable: Housing Condition	**Number Moving**	**Activity Amount**	**Activity Level**
Chi-Square	2.872	7.381	9.683
Df	2	2	2
Asymp. Sig.	.238	.025	.008

Table 13b. Experiment II Phase B Home Cage Activity Kruskal Wallis Test

Kruskal-Wallis Test	Housing Condition	N	Mean Rank
Number of Animals Moving	Non-Enriched	10	15.50
	Enriched	10	15.50
	Super-Enriched	10	15.50
	Total	30	
Amount of Activity	Non-Enriched	10	6.90
	Enriched	10	18.40
	Super-Enriched	10	21.20
	Total	30	
Level of Activity	Non-Enriched	10	6.00
	Enriched	10	17.00
	Super-Enriched	10	23.50
	Total	30	
Grouping Variable: Housing Condition	**Number Moving**	**Activity Amount**	**Activity Level**
Chi-Square	.000	16.130	21.909
Df	2	2	2
Asymp. Sig.	1.000	.000	.000

Table 13c. Experiment II Phase A2 Home Cage Activity Kruskal Wallis Test

Kruskal-Wallis Test	Housing Condition	N	Mean Rank
Number of Animals Moving	Non-Enriched	14	21.50
	Enriched	14	21.50
	Super-Enriched	14	21.50
	Total	42	
Amount of Activity	Non-Enriched	14	18.25
	Enriched	14	17.39
	Super-Enriched	14	28.86
	Total	42	
Level of Activity	Non-Enriched	14	13.57
	Enriched	14	17.07
	Super-Enriched	14	33.86
	Total	42	
Grouping Variable: Housing Condition	**Number Moving**	**Activity Amount**	**Activity Level**
Chi-Square	.000	8.084	23.016
Df	2	2	2
Asymp. Sig.	1.000	.018	.000

Table 14a. Experiment II Open Field MANOVA

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
HACTV1	Contrast	312134429.056	2	156067214.528	21.539	.000	.566	1.000
	Error	239113003.917	33	7245848.604				
VACTV1	Contrast	1886723.389	2	943361.694	13.313	.000	.447	.996
	Error	2338420.917	33	70861.240				
HACTV2	Contrast	981469735.509	2	490734867.754	47.804	.000	.743	1.000
	Error	338764221.063	33	10265582.456				
VACTV2	Contrast	4985027.722	2	2492513.861	28.925	.000	.637	1.000
	Error	2843622.167	33	86170.369				
HACTV3	Contrast	320948500.167	2	160474250.083	11.052	.000	.401	.986
	Error	479153200.583	33	14519793.957				
VACTV3	Contrast	1105075.500	2	552537.750	3.515	.041	.176	.615
	Error	5186863.250	33	157177.674				
HACTV4	Contrast	545111057.056	2	272555528.528	28.730	.000	.635	1.000
	Error	313063811.500	33	9486782.167				
VACTV4	Contrast	2775891.167	2	1387945.583	7.744	.002	.319	.930
	Error	5914273.583	33	179220.412				
HACTV5	Contrast	592681121.167	2	296340560.583	24.658	.000	.599	1.000
	Error	396596836.833	33	12018085.965				
VACTV5	Contrast	6365604.389	2	3182802.194	12.984	.000	.440	.995
	Error	8089353.500	33	245131.924				
HACTV6	Contrast	435084650.889	2	217542325.444	7.528	.002	.313	.923
	Error	953575766.667	33	28896235.354				
VACTV6	Contrast	19243272.167	2	9621636.083	5.152	.011	.238	.789
	Error	61626384.583	33	1867466.199				

Table 14b. Experiment II OF 1 Horizontal Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	114402936.239	11	10400266.931	63.488	.000	.658	1.000
Time * Housing	6887849.410	22	313084.064	1.911	.009	.104	.988
Error(time)	59464666.878	363	163814.509				
Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	26011202.421	2	13005601.211	21.539	.000	.566	1.000
Error	19926083.660	33	603820.717				

Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	128.118	91.577	.513	-102.859	359.095
	Super-Enriched	572.625(*)	91.577	.000	341.648	803.602
Enriched	Non-Enriched	-128.118	91.577	.513	-359.095	102.859
	Super-Enriched	444.507(*)	91.577	.000	213.530	675.484
Super-Enriched	Non-Enriched	-572.625(*)	91.577	.000	-803.602	-341.648
	Enriched	-444.507(*)	91.577	.000	-675.484	-213.530

Table 14c. Experiment II OF 1 Vertical Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	778613.189	11	70783.017	48.803	.000	.597	1.000
Time*Housing	64743.379	22	2942.881	2.029	.004	.110	.992
Error(time)	526485.431	363	1450.373				
Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	157226.949	2	78613.475	13.313	.000	.447	.996
Error	194868.410	33	5905.103				

Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	7.674	9.056	1.000	-15.168	30.515
	Super-Enriched	43.757(*)	9.056	.000	20.915	66.599
Enriched	Non-Enriched	-7.674	9.056	1.000	-30.515	15.168
	Super-Enriched	36.083(*)	9.056	.001	13.242	58.925
Super-Enriched	Non-Enriched	-43.757(*)	9.056	.000	-66.599	-20.915
	Enriched	-36.083(*)	9.056	.001	-58.925	-13.242

Table 14d. Experiment II OF 2 Horizontal Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	132232292.859	11	12021117.533	49.272	.000	.599	1.000
Time*Housing	10000051.356	22	454547.789	1.863	.011	.101	.985
Error(time)	88562655.201	363	243974.257				

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	75442886.255	2	37721443.127	47.870	.000	.744	1.000
Error	26004019.049	33	788000.577				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	303.757(*)	104.616	.020	39.894	567.620
	Super-Enriched	998.438(*)	104.616	.000	734.574	1262.301
Enriched	Non-Enriched	-303.757(*)	104.616	.020	-567.620	-39.894
	Super-Enriched	694.681(*)	104.616	.000	430.817	958.544
Super-Enriched	Non-Enriched	-998.438(*)	104.616	.000	-1262.301	-734.574
	Enriched	-694.681(*)	104.616	.000	-958.544	-430.817

Table 14e. Experiment II OF 2 Vertical Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	1000437.944	11	90948.904	35.383	.000	.517	1.000
Time*Housing	102204.347	22	4645.652	1.807	.015	.099	.982
Error(time)	933064.208	363	2570.425				

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	413552.542	2	206776.271	28.744	.000	.635	1.000
Error	237392.625	33	7193.716				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	18.938	9.996	.201	-6.274	44.149
	Super-Enriched	73.021(*)	9.996	.000	47.810	98.232
Enriched	Non-Enriched	-18.938	9.996	.201	-44.149	6.274
	Super-Enriched	54.083(*)	9.996	.000	28.872	79.294
Super-Enriched	Non-Enriched	-73.021(*)	9.996	.000	-98.232	-47.810
	Enriched	-54.083(*)	9.996	.000	-79.294	-28.872

Table 14f. Experiment II OF 3 Horizontal Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	137746139.618	11	12522376.329	44.932	.000	.577	1.000
Time*Housing	7174199.264	22	326099.967	1.170	.272	.066	.860
Error(time)	101166300.368	363	278695.042				

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	26745708.347	2	13372854.174	11.052	.000	.401	.986
Error	39929433.382	33	1209982.830				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	114.069	129.635	1.000	-212.898	441.037
	Super-Enriched	575.535(*)	129.635	.000	248.567	902.503
Enriched	Non-Enriched	-114.069	129.635	1.000	-441.037	212.898
	Super-Enriched	461.465(*)	129.635	.003	134.497	788.433
Super-Enriched	Non-Enriched	-575.535(*)	129.635	.000	-902.503	-248.567
	Enriched	-461.465(*)	129.635	.003	-788.433	-134.497

Table 14g. Experiment II OF 3 Vertical Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	967896.019	11	87990.547	33.911	.000	.507	1.000
Time*Housing	83295.329	22	3786.151	1.459	.085	.081	.941
Error(time)	941886.319	363	2594.728				

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	63195.449	2	31597.725	2.355	.111	.125	.443
Error	442751.514	33	13416.713				

Table 14h. Experiment II OF 4 Horizontal Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	162860736.546	11	14805521.504	60.282	.000	.646	1.000
Time*Housing	9763128.745	22	443778.579	1.807	.015	.099	.982
Error(time)	89154933.208	363	245605.877				

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	45425921.421	2	22712960.711	28.730	.000	.635	1.000
Error	26088650.958	33	790565.181				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	136.243	104.786	.608	-128.049	400.535
	Super-Enriched	745.813(*)	104.786	.000	481.520	1010.105
Enriched	Non-Enriched	-136.243	104.786	.608	-400.535	128.049
	Super-Enriched	609.569(*)	104.786	.000	345.277	873.862
Super-Enriched	Non-Enriched	-745.813(*)	104.786	.000	-1010.105	-481.520
	Enriched	-609.569(*)	104.786	.000	-873.862	-345.277

Table 14i. Experiment II OF 4 Vertical Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	1569017.951	11	142637.996	32.367	.000	.495	1.000
Time*Housing	175520.514	22	7978.205	1.810	.015	.099	.982
Error(time)	1599704.451	363	4406.899				

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	231324.264	2	115662.132	7.744	.002	.319	.930
Error	492856.132	33	14935.034				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	6.326	14.402	1.000	-30.000	42.652
	Super-Enriched	51.944(*)	14.402	.003	15.618	88.271
Enriched	Non-Enriched	-6.326	14.402	1.000	-42.652	30.000
	Super-Enriched	45.618(*)	14.402	.010	9.292	81.944
Super-Enriched	Non-Enriched	-51.944(*)	14.402	.003	-88.271	-15.618
	Enriched	-45.618(*)	14.402	.010	-81.944	-9.292

Table 14j. Experiment II OF 5 Horizontal Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	184771724.081	11	16797429.462	67.661	.000	.672	1.000
Time*Housing	8715167.370	22	396143.971	1.596	.044	.088	.962
Error(time)	90118067.965	363	248259.140				

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	48421653.407	2	24210826.704	25.648	.000	.609	1.000
Error	31150828.451	33	943964.499				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	340.667(*)	114.502	.016	51.869	629.464
	Super-Enriched	816.361(*)	114.502	.000	527.564	1105.159
Enriched	Non-Enriched	-340.667(*)	114.502	.016	-629.464	-51.869
	Super-Enriched	475.694(*)	114.502	.001	186.897	764.492
Super-Enriched	Non-Enriched	-816.361(*)	114.502	.000	-1105.159	-527.564
	Enriched	-475.694(*)	114.502	.001	-764.492	-186.897

Table 14k. Experiment II OF 5 Vertical Activity

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	1792026.380	11	162911.489	42.938	.000	.565	1.000
Time*Housing	141509.079	22	6432.231	1.695	.027	.093	.973
Error(time)	1377246.708	363	3794.068				

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	530467.032	2	265233.516	12.984	.000	.440	.995
Error	674112.792	33	20427.660				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.

(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	41.215	16.844	.060	-1.269	83.699
	Super-Enriched	85.813(*)	16.844	.000	43.329	128.296
Enriched	Non-Enriched	-41.215	16.844	.060	-83.699	1.269
	Super-Enriched	44.597(*)	16.844	.037	2.113	87.081
Super-Enriched	Non-Enriched	-85.813(*)	16.844	.000	-128.296	-43.329
	Enriched	-44.597(*)	16.844	.037	-87.081	-2.113

Table 14l. Experiment II OF 6 Horizontal Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	164296458.907	11	14936041.719	56.915	.000	.633	1.000
Time*Housing	10045257.981	22	456602.636	1.740	.021	.095	.977
Error(time)	95260863.611	363	262426.622				
Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	36257054.241	2	18128527.120	7.528	.002	.313	.923
Error	79464647.222	33	2408019.613				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	49.653	182.879	1.000	-411.607	510.912
	Super-Enriched	637.875(*)	182.879	.004	176.615	1099.135
Enriched	Non-Enriched	-49.653	182.879	1.000	-510.912	411.607
	Super-Enriched	588.222(*)	182.879	.009	126.963	1049.482
Super-Enriched	Non-Enriched	-637.875(*)	182.879	.004	-1099.135	-176.615
	Enriched	-588.222(*)	182.879	.009	-1049.482	-126.963

Table 14m. Experiment II OF 6 Vertical Activity

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	1486288.692	11	135117.154	37.761	.000	.534	1.000
Time*Housing	115526.134	22	5251.188	1.468	.081	.082	.942
Error(time)	1298903.090	363	3578.245				
Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	439095.421	2	219547.711	6.497	.004	.283	.879
Error	1115203.493	33	33794.045				

Between -Subjects Effects Post Hoc Pairwise Comparisons - Adjustment for multiple comparisons: Bonferroni.						
(I) Housing	(J) Housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched	Enriched	-5.792	21.665	1.000	-60.435	48.851
	Super-Enriched	64.549(*)	21.665	.016	9.905	119.192
Enriched	Non-Enriched	5.792	21.665	1.000	-48.851	60.435
	Super-Enriched	70.340(*)	21.665	.008	15.697	124.983
Super-Enriched	Non-Enriched	-64.549(*)	21.665	.016	-119.192	-9.905
	Enriched	-70.340(*)	21.665	.008	-124.983	-15.697

Table 15. Experiment III Timeline

Date	Day of Study		Measure/Activity
7 Apr	Day 1 - **Phase 1**		New Housing – FC & BW
8 Apr	Day 2		HCA
9 Apr	Day 3		FC
10 Apr	Day 4		
11 Apr	Day 5		FC & BW
12 Apr	Day 6		
13 Apr	Day 7		HCA & FC
14 Apr	Day 8		
15 Apr	Day 9		FC
16 Apr	Day 10		BW
17 Apr	Day 11		FC
18 Apr	Day 12		
19 Apr	Day 13		FC & OF
20 Apr	Day 14		HCA & BW
21 Apr	Day 15 **- Phase 2**	Day 1	FC
22 Apr	Day 17	Day 2	HCA
23 Apr	Day 18	Day 3	FC & OF
24 Apr	Day 19	Day 4	
25 Apr	Day 20	Day 5	FC & BW
26 Apr	Day 21	Day 6	
27 Apr	Day 22	Day 7	HCA
28 Apr	Day 23	Day 8	
29 Apr	Day 24	Day 9	FC
30 Apr	Day 25	Day 10	BW
1 May	Day 26	Day 11	FC
2 May	Day 27	Day 12	
3 May	Day 28	Day 13	FC & OF
4 May	Day 29	Day 14	HCA & BW
5 May	Day 30 **- Phase 3**	Day 1	FC
6 May	Day 31	Day 2	HCA
7 May	Day 32	Day 3	FC & OF
8 May	Day 33	Day 4	
9 May	Day 34	Day 5	FC & BW
10 May	Day 35	Day 6	
11 May	Day 36	Day 7	HCA & FC
12 May	Day 37	Day 8	
13 May	Day 38	Day 9	FC
14 May	Day 39	Day 10	BW
15 May	Day 40	Day 11	FC
16 May	Day 41	Day 12	
17 May	Day 41	Day 13	FC
18 May	Day 43	Day 14	HCA & BW
19 May	Day 44	Day 15	FC
20 May	Day 45	Day 16	HCA & OF
21 May	Day 46	Day 17	FC
22 May	Day 47	Day 18	
23 May	Day 48	Day 19	FC & BW
24 May	Day 49	Day 20	HCA
25 May	Day 50	Day 21	Sacrifice

BW = Body Weight Measurement
FC = Food Consumption
HCA = Home Cage Activity
OF = Open Field (locomotor)

Table 16. Experiment III Body Weight ANCOVA

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	7415.795	10	741.580	4.156	.000	.090	.998
Time * Housing	21584.507	10	2158.451	12.096	.000	.224	1.000
Time * Sex	207205.655	10	20720.566	116.116	.000	.734	1.000
Time*housing * Sex	1678.393	10	167.839	.941	.496	.022	.501
Error(time)	74947.566	420	178.447				

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	76634.507	1	76634.507	15.293	.000	.267	.969
Sex	401682.612	1	401682.612	80.161	.000	.656	1.000
Housing * Sex	1618.899	1	1618.899	.323	.573	.008	.086
Error	210461.122	42	5010.979				

Post-Hoc Pairwise Comparisons – No Adjustment for Multiple Comparisons

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Non-Enriched Males	Non-Enriched Females	62.589(*)	9.955	.000	42.500	82.679
	Super-Enriched Males	21.502(*)	9.123	.023	3.092	39.913
	Super-Enriched Females	91.195(*)	10.448	.000	70.110	112.281
Non-Enriched Females	Non-Enriched Males	-62.589(*)	9.955	.000	-82.679	-42.500
	Super-Enriched Males	-41.087(*)	9.059	.000	-59.369	-22.805
	Super-Enriched Females	28.606(*)	8.773	.002	10.902	46.310
Super-Enriched Males	Non-Enriched Males	-21.502(*)	9.123	.023	-39.913	-3.092
	Non-Enriched Females	41.087(*)	9.059	.000	22.805	59.369
	Super-Enriched Females	69.693(*)	9.388	.000	50.746	88.640
Super-Enriched Females	Non-Enriched Males	-91.195(*)	10.448	.000	-112.281	-70.110
	Non-Enriched Females	-28.606(*)	8.773	.002	-46.310	-10.902
	Super-Enriched Males	-69.693(*)	9.388	.000	-88.640	-50.746

Table 17. Experiment III Body Mass Index

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	797.011	1	797.011	15.458	.000	.264	.970
Sex	756.578	1	756.578	14.674	.000	.254	.963
Housing * Sex	3.204	1	3.204	.062	.804	.001	.057
Error	2217.048	43	51.559				

Post-Hoc Analyses - Tukey HSD						
(I) ExpGrp	(J) ExpGrp	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched Males	Non-Enriched Females	8.55259(*)	2.997	.032	.54254	16.56264
	Super-Enriched Males	8.76437(*)	2.997	.027	.75431	16.77442
	Super-Enriched Females	16.27180(*)	2.997	.000	8.26175	24.28185
Non-Enriched Females	Non-Enriched Males	-8.55259(*)	2.997	.032	-16.56264	-.54254
	Super-Enriched Males	.21178	2.931	1.000	-7.62221	8.04576
	Super-Enriched Females	7.71921	2.931	.055	-.11478	15.55319
Super-Enriched Males	Non-Enriched Males	-8.76437(*)	2.997	.027	-16.77442	-.75431
	Non-Enriched Females	-.21178	2.931	1.000	-8.04576	7.62221
	Super-Enriched Females	7.50743	2.931	.065	-.32655	15.34142
Super-Enriched Females	Non-Enriched Males	-16.27180(*)	2.997	.000	-24.28185	-8.26175
	Non-Enriched Females	-7.71921	2.931	.055	-15.55319	.11478
	Super-Enriched Males	-7.50743	2.931	.065	-15.34142	.32655

Table 18. Experiment III Lee Index Tests of Between-Subjects Effects

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	1448.283	1	1448.283	8.190	.006	.160	.799
Sex	474.191	1	474.191	2.682	.109	.059	.360
Housing * Sex	76.057	1	76.057	.430	.515	.010	.098
Error	7603.976	43	176.837				

Post-Hoc Analyses - Tukey HSD						
(I) ExpGrp	(J) ExpGrp	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Enriched Males	Non-Enriched Females	-3.81120	5.550896	.902	-18.64553	11.02314
	Super-Enriched Males	13.65605	5.550896	.081	-1.17829	28.49039
	Super-Enriched Females	4.75285	5.550896	.827	-10.08149	19.58719
Non-Enriched Females	Non-Enriched Males	3.81120	5.550896	.902	-11.02314	18.64553
	Super-Enriched Males	17.46725(*)	5.428883	.013	2.95898	31.97552
	Super-Enriched Females	8.56405	5.428883	.402	-5.94422	23.07231
Super-Enriched Males	Non-Enriched Males	-13.65605	5.550896	.081	-28.49039	1.17829
	Non-Enriched Females	-17.46725(*)	5.428883	.013	-31.97552	-2.95898
	Super-Enriched Females	-8.90320	5.428883	.368	-23.41147	5.60507
Super-Enriched Females	Non-Enriched Males	-4.75285	5.550896	.827	-19.58719	10.08149
	Non-Enriched Females	-8.56405	5.428883	.402	-23.07231	5.94422
	Super-Enriched Males	8.90320	5.428883	.368	-5.60507	23.41147

Table 19a. Experiment III Phase A1 Daily Grams Consumed

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	89.093	1	89.093	69.175	.000	.617	1.000
Sex	55.985	1	55.985	43.469	.000	.503	1.000
Housing * Sex	.142	1	.142	.110	.742	.003	.062
Error	55.381	43	1.288				

Post-Hoc Analyses - Tukey HSD						
(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	2.0745(*)	.47372	.000	.8085	3.3404
	Male Super-Enriched	2.6456(*)	.47372	.000	1.3797	3.9116
	Female Super-Enriched	4.9399(*)	.47372	.000	3.6739	6.2059
Female Non-Enriched	Male Non-Enriched	-2.0745(*)	.47372	.000	-3.3404	-.8085
	Male Super-Enriched	.5712	.46331	.610	-.6670	1.8093
	Female Super-Enriched	2.8655(*)	.46331	.000	1.6273	4.1036
Male Super-Enriched	Male Non-Enriched	-2.6456(*)	.47372	.000	-3.9116	-1.3797
	Female Non-Enriched	-.5712	.46331	.610	-1.8093	.6670
	Female Super-Enriched	2.2943(*)	.46331	.000	1.0561	3.5324
Female Super-Enriched	Male Non-Enriched	-4.9399(*)	.47372	.000	-6.2059	-3.6739
	Female Non-Enriched	-2.8655(*)	.46331	.000	-4.1036	-1.6273
	Male Super-Enriched	-2.2943(*)	.46331	.000	-3.5324	-1.0561

Table 19b. Experiment III Phase A1 Daily Calories Consumed

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	38.809	1	38.809	69.175	.000	.617	1.000
Sex	24.387	1	24.387	43.469	.000	.503	1.000
Housing * Sex	.062	1	.062	.110	.742	.003	.062
Error	24.124	43	.561				

Post-Hoc Analyses - Tukey HSD						
(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	1.369143(*)	.3126565	.000	.533593	2.204693
	Male Super-Enriched	1.746129(*)	.3126565	.000	.910578	2.581679
	Female Super-Enriched	3.260357(*)	.3126565	.000	2.424807	4.095907
Female Non-Enriched	Male Non-Enriched	-1.369143(*)	.3126565	.000	-2.204693	-.533593
	Male Super-Enriched	.376986	.3057841	.610	-.440198	1.194170
	Female Super-Enriched	1.891214(*)	.3057841	.000	1.074030	2.708398
Male Super-Enriched	Male Non-Enriched	-1.746129(*)	.3126565	.000	-2.581679	-.910578
	Female Non-Enriched	-.376986	.3057841	.610	-1.194170	.440198
	Female Super-Enriched	1.514229(*)	.3057841	.000	.697044	2.331413
Female Super-Enriched	Male Non-Enriched	-3.260357(*)	.3126565	.000	-4.095907	-2.424807
	Female Non-Enriched	-1.891214(*)	.3057841	.000	-2.708398	-1.074030
	Male Super-Enriched	-1.514229(*)	.3057841	.000	-2.331413	-.697044

Table 19c. Experiment III Phase B Daily Grams Consumed

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	154.180	1	154.180	24.173	.000	.360	.998
Sex	464.934	1	464.934	72.894	.000	.629	1.000
Housing * Sex	13.099	1	13.099	2.054	.159	.046	.289
Error	274.264	43	6.378				

Post-Hoc Analyses - Tukey HSD						
(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	5.2383(*)	1.05421	.000	2.4210	8.0556
	Male Super-Enriched	2.5684	1.05421	.085	-.2489	5.3857
	Female Super-Enriched	9.9198(*)	1.05421	.000	7.1025	12.7371
Female Non-Enriched	Male Non-Enriched	-5.2383(*)	1.05421	.000	-8.0556	-2.4210
	Male Super-Enriched	-2.6699	1.03104	.061	-5.4252	.0855
	Female Super-Enriched	4.6815(*)	1.03104	.000	1.9262	7.4369
Male Super-Enriched	Male Non-Enriched	-2.5684	1.05421	.085	-5.3857	.2489
	Female Non-Enriched	2.6699	1.03104	.061	-.0855	5.4252
	Female Super-Enriched	7.3514(*)	1.03104	.000	4.5961	10.1068
Female Super-Enriched	Male Non-Enriched	-9.9198(*)	1.05421	.000	-12.7371	-7.1025
	Female Non-Enriched	-4.6815(*)	1.03104	.000	-7.4369	-1.9262
	Male Super-Enriched	-7.3514(*)	1.03104	.000	-10.1068	-4.5961

Table 19d. Experiment III Phase B Daily Calories Consumed

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	3746.951	1	3746.951	40.851	.000	.487	1.000
Sex	398.260	1	398.260	4.342	.043	.092	.531
Housing * Sex	.647	1	.647	.007	.933	.000	.051
Error	3944.101	43	91.723				

Post-Hoc Analyses - Tukey HSD

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	5.591212	3.9977603	.507	-5.092492	16.274915
	Male Super-Enriched	17.635337(*)	3.9977603	.000	6.951634	28.319041
	Female Super-Enriched	23.696181(*)	3.9977603	.000	13.012477	34.379884
Female Non-Enriched	Male Non-Enriched	-5.591212	3.9977603	.507	-16.274915	5.092492
	Male Super-Enriched	12.044126(*)	3.9098867	.018	1.595258	22.492994
	Female Super-Enriched	18.104969(*)	3.9098867	.000	7.656101	28.553837
Male Super-Enriched	Male Non-Enriched	-17.635337(*)	3.9977603	.000	-28.319041	-6.951634
	Female Non-Enriched	-12.044126(*)	3.9098867	.018	-22.492994	-1.595258
	Female Super-Enriched	6.060843	3.9098867	.417	-4.388025	16.509711
Female Super-Enriched	Male Non-Enriched	-23.696181(*)	3.9977603	.000	-34.379884	-13.012477
	Female Non-Enriched	-18.104969(*)	3.9098867	.000	-28.553837	-7.656101
	Male Super-Enriched	-6.060843	3.9098867	.417	-16.509711	4.388025

Table 19e. Experiment III Phase B Daily Standard Chow Grams Consumed

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	.001	1	.001	.000	.990	.000	.050
Sex	439.339	1	439.339	150.316	.000	.778	1.000
Housing * Sex	14.610	1	14.610	4.999	.031	.104	.589
Error	125.679	43	2.923				

Post-Hoc Analyses - Tukey HSD

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	5.0032(*)	.71363	.000	3.0961	6.9104
	Male Super-Enriched	-1.1225	.71363	.404	-3.0296	.7847
	Female Super-Enriched	6.1125(*)	.71363	.000	4.2054	8.0197
Female Non-Enriched	Male Non-Enriched	-5.0032(*)	.71363	.000	-6.9104	-3.0961
	Male Super-Enriched	-6.1257(*)	.69795	.000	-7.9909	-4.2605
	Female Super-Enriched	1.1093	.69795	.395	-.7559	2.9745
Male Super-Enriched	Male Non-Enriched	1.1225	.71363	.404	-.7847	3.0296
	Female Non-Enriched	6.1257(*)	.69795	.000	4.2605	7.9909
	Female Super-Enriched	7.2350(*)	.69795	.000	5.3698	9.1002
Female Super-Enriched	Male Non-Enriched	-6.1125(*)	.71363	.000	-8.0197	-4.2054
	Female Non-Enriched	-1.1093	.69795	.395	-2.9745	.7559
	Male Super-Enriched	-7.2350(*)	.69795	.000	-9.1002	-5.3698

Table 19f. Experiment III Phase B Daily Standard Chow Calories Consumed

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	.000	1	.000	.000	.990	.000	.050
Sex	191.376	1	191.376	150.316	.000	.778	1.000
Housing * Sex	6.364	1	6.364	4.999	.031	.104	.589
Error	54.746	43	1.273				

Post-Hoc Analyses - Tukey HSD

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	3.30214(*)	.470998	.000	2.04344	4.56085
	Male Super-Enriched	-.74083	.470998	.404	-1.99953	.51788
	Female Super-Enriched	4.03427(*)	.470998	.000	2.77557	5.29298
Female Non-Enriched	Male Non-Enriched	-3.30214(*)	.470998	.000	-4.56085	-2.04344
	Male Super-Enriched	-4.04297(*)	.460645	.000	-5.27401	-2.81193
	Female Super-Enriched	.73213	.460645	.395	-.49891	1.96317
Male Super-Enriched	Male Non-Enriched	.74083	.470998	.404	-.51788	1.99953
	Female Non-Enriched	4.04297(*)	.460645	.000	2.81193	5.27401
	Female Super-Enriched	4.77510(*)	.460645	.000	3.54406	6.00614
Female Super-Enriched	Male Non-Enriched	-4.03427(*)	.470998	.000	-5.29298	-2.77557
	Female Non-Enriched	-.73213	.460645	.395	-1.96317	.49891
	Male Super-Enriched	-4.77510(*)	.460645	.000	-6.00614	-3.54406

Table 19g. Experiment III Phase B Daily Cookie Grams Consumed

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	26.180	1	26.180	17.454	.000	.289	.983
Sex	3.478	1	3.478	2.319	.135	.051	.319
Housing * Sex	2.935	1	2.935	1.957	.169	.044	.277
Error	64.499	43	1.500				

Post-Hoc Analyses - Tukey HSD

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	-1.0446	.51123	.188	-2.4109	.3216
	Male Super-Enriched	.9936	.51123	.226	-.3727	2.3598
	Female Super-Enriched	.9493	.51123	.262	-.4169	2.3155
Female Non-Enriched	Male Non-Enriched	1.0446	.51123	.188	-.3216	2.4109
	Male Super-Enriched	2.0382(*)	.50000	.001	.7020	3.3744
	Female Super-Enriched	1.9939(*)	.50000	.001	.6577	3.3301
Male Super-Enriched	Male Non-Enriched	-.9936	.51123	.226	-2.3598	.3727
	Female Non-Enriched	-2.0382(*)	.50000	.001	-3.3744	-.7020
	Female Super-Enriched	-.0443	.50000	1.000	-1.3805	1.2919
Female Super-Enriched	Male Non-Enriched	-.9493	.51123	.262	-2.3155	.4169
	Female Non-Enriched	-1.9939(*)	.50000	.001	-3.3301	-.6577
	Male Super-Enriched	.0443	.50000	1.000	-1.2919	1.3805

Table 19h. Experiment III Phase B Daily Cookie Calories Consumed

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	816.083	1	816.083	25.938	.000	.376	.999
Sex	113.326	1	113.326	3.602	.064	.077	.458
Housing * Sex	54.247	1	54.247	1.724	.196	.039	.250
Error	1352.915	43	31.463				

Post-Hoc Analyses - Tukey HSD

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	-5.258010	2.3414124	.127	-11.5152	.999232
	Male Super-Enriched	6.189620	2.3414124	.053	-.067622	12.446863
	Female Super-Enriched	5.232004	2.3414124	.130	-1.02523	11.489246
Female Non-Enriched	Male Non-Enriched	5.258010	2.3414124	.127	-.999232	11.515253
	Male Super-Enriched	11.447631(*)	2.2899465	.000	5.327927	17.567334
	Female Super-Enriched	10.490014(*)	2.2899465	.000	4.370310	16.609718
Male Super-Enriched	Male Non-Enriched	-6.189620	2.3414124	.053	-12.4468	.067622
	Female Non-Enriched	-11.447631(*)	2.2899465	.000	-17.5673	-5.327927
	Female Super-Enriched	-.957617	2.2899465	.975	-7.07732	5.162087
Female Super-Enriched	Male Non-Enriched	-5.232004	2.3414124	.130	-11.4892	1.025239
	Female Non-Enriched	-10.490014(*)	2.2899465	.000	-16.6097	-4.370310
	Male Super-Enriched	.957617	2.2899465	.975	-5.1620	7.077321

Table 19i. Experiment III Phase B Daily Chip Grams Consumed

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	61.317	1	61.317	21.237	.000	.326	.995
Sex	8.525	1	8.525	2.953	.093	.063	.390
Housing * Sex	5.584	1	5.584	1.934	.171	.042	.275
Error	127.040	44	2.887				

Post-Hoc Analyses - Tukey HSD

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	1.5250	.69370	.140	-.3272	3.3772
	Male Super-Enriched	2.9426(*)	.69370	.001	1.0904	4.7948
	Female Super-Enriched	3.1033(*)	.69370	.000	1.2512	4.9555
Female Non-Enriched	Male Non-Enriched	-1.5250	.69370	.140	-3.3772	.3272
	Male Super-Enriched	1.4176	.69370	.188	-.4346	3.2698
	Female Super-Enriched	1.5783	.69370	.120	-.2738	3.4305
Male Super-Enriched	Male Non-Enriched	-2.9426(*)	.69370	.001	-4.7948	-1.0904
	Female Non-Enriched	-1.4176	.69370	.188	-3.2698	.4346
	Female Super-Enriched	.1607	.69370	.996	-1.6915	2.0129
Female Super-Enriched	Male Non-Enriched	-3.1033(*)	.69370	.000	-4.9555	-1.2512
	Female Non-Enriched	-1.5783	.69370	.120	-3.4305	.2738
	Male Super-Enriched	-.1607	.69370	.996	-2.0129	1.6915

Table 19j. Experiment III Phase B Daily Chip Calories Consumed

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	1066.680	1	1066.680	16.437	.000	.277	.977
Sex	281.168	1	281.168	4.333	.043	.092	.530
Housing * Sex	82.513	1	82.513	1.271	.266	.029	.197
Error	2790.474	43	64.895				

Post-Hoc Analyses - Tukey HSD

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	7.54708	3.362650	.128	-1.43934	16.53350
	Male Super-Enriched	12.18655(*)	3.362650	.004	3.20012	21.17297
	Female Super-Enriched	14.42991(*)	3.362650	.001	5.44348	23.41633
Female Non-Enriched	Male Non-Enriched	-7.54708	3.362650	.128	-16.53350	1.43934
	Male Super-Enriched	4.63947	3.288737	.500	-4.14943	13.42836
	Female Super-Enriched	6.88283	3.288737	.172	-1.90607	15.67172
Male Super-Enriched	Male Non-Enriched	-12.18655(*)	3.362650	.004	-21.17297	-3.20012
	Female Non-Enriched	-4.63947	3.288737	.500	-13.42836	4.14943
	Female Super-Enriched	2.24336	3.288737	.903	-6.54553	11.03225
Female Super-Enriched	Male Non-Enriched	-14.42991(*)	3.362650	.001	-23.41633	-5.44348
	Female Non-Enriched	-6.88283	3.288737	.172	-15.67172	1.90607
	Male Super-Enriched	-2.24336	3.288737	.903	-11.03225	6.54553

Table 19k. Experiment III Phase A2 Daily Grams Consumed

Tests of Between -Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	156.022	1	156.022	59.788	.000	.582	1.000
Sex	768.156	1	768.156	294.360	.000	.873	1.000
Housing * Sex	6.164	1	6.164	2.362	.132	.052	.324
Error	112.212	43	2.610				

Post-Hoc Analyses - Tukey HSD

(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	7.3664(*)	.67431	.000	5.5644	9.1685
	Male Super-Enriched	2.9218(*)	.67431	.000	1.1197	4.7238
	Female Super-Enriched	11.7378(*)	.67431	.000	9.9357	13.5398
Female Non-Enriched	Male Non-Enriched	-7.3664(*)	.67431	.000	-9.1685	-5.5644
	Male Super-Enriched	-4.4447(*)	.65949	.000	-6.2071	-2.6822
	Female Super-Enriched	4.3713(*)	.65949	.000	2.6089	6.1338
Male Super-Enriched	Male Non-Enriched	-2.9218(*)	.67431	.000	-4.7238	-1.1197
	Female Non-Enriched	4.4447(*)	.65949	.000	2.6822	6.2071
	Female Super-Enriched	8.8160(*)	.65949	.000	7.0536	10.5784
Female Super-Enriched	Male Non-Enriched	-11.7378(*)	.67431	.000	-13.5398	-9.9357
	Female Non-Enriched	-4.3713(*)	.65949	.000	-6.1338	-2.6089
	Male Super-Enriched	-8.8160(*)	.65949	.000	-10.5784	-7.0536

Table 19l. Experiment III Phase A2 Daily Calories Consumed

Tests of Between -Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Housing	67.963	1	67.963	59.788	.000	.582	1.000
Sex	334.609	1	334.609	294.360	.000	.873	1.000
Housing * Sex	2.685	1	2.685	2.362	.132	.052	.324
Error	48.880	43	1.137				

Post-Hoc Analyses - Tukey HSD						
(I) Expgrp	(J) Expgrp	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Male Non-Enriched	Female Non-Enriched	4.86185(*)	.445047	.000	3.67250	6.05120
	Male Super-Enriched	1.92837(*)	.445047	.000	.73902	3.11772
	Female Super-Enriched	7.74693(*)	.445047	.000	6.55758	8.93628
Female Non-Enriched	Male Non-Enriched	-4.86185(*)	.445047	.000	-6.05120	-3.67250
	Male Super-Enriched	-2.93348(*)	.435265	.000	-4.09669	-1.77027
	Female Super-Enriched	2.88508(*)	.435265	.000	1.72187	4.04829
Male Super-Enriched	Male Non-Enriched	-1.92837(*)	.445047	.000	-3.11772	-.73902
	Female Non-Enriched	2.93348(*)	.435265	.000	1.77027	4.09669
	Female Super-Enriched	5.81856(*)	.435265	.000	4.65535	6.98177
Female Super-Enriched	Male Non-Enriched	-7.74693(*)	.445047	.000	-8.93628	-6.55758
	Female Non-Enriched	-2.88508(*)	.435265	.000	-4.04829	-1.72187
	Male Super-Enriched	-5.81856(*)	.435265	.000	-6.98177	-4.65535

Table 20a. Experiment III Phase A1 Home Cage Activity Kruskal-Wallis

Kruskal-Wallis Test	Housing Condition	N	Mean Rank
Number Moving	Non-Enriched Male	9	17.17
	Non-Enriched Female	9	14.61
	Super-Enriched Male	9	18.94
	Super-Enriched Female	9	23.28
	Total	36	
Amount Activity	Non-Enriched Male	9	12.83
	Non-Enriched Female	9	12.67
	Super-Enriched Male	9	26.67
	Super-Enriched Female	9	21.83
	Total	36	
Level Activity	Non-Enriched Male	9	13.67
	Non-Enriched Female	9	13.50
	Super-Enriched Male	9	27.22
	Super-Enriched Female	9	19.61
	Total	36	
Grouping Variable: Housing Condition	**Number Moving**	**Activity Amount**	**Activity Level**
Chi-Square	3.589	12.470	11.070
df	3	3	3
Asymp. Sig.	.309	.006	.011

Table 20b. Experiment III Phase B Home Cage Activity Kruskal-Wallis

Kruskal-Wallis Test	Housing Condition	N	Mean Rank
Number Moving	Non-Enriched Male	12	15.50
	Non-Enriched Female	14	27.50
	Super-Enriched Male	10	27.50
	Super-Enriched Female	12	27.50
	Total	48	
Amount Activity	Non-Enriched Male	12	17.63
	Non-Enriched Female	14	15.54
	Super-Enriched Male	10	30.00
	Super-Enriched Female	12	37.25
	Total	48	
Level Activity	Non-Enriched Male	12	16.25
	Non-Enriched Female	14	18.11
	Super-Enriched Male	10	30.25
	Super-Enriched Female	12	35.42
	Total	48	
Grouping Variable: Housing Condition	**Number Moving**	**Activity Amount**	**Activity Level**
Chi-Square	20.143	22.786	17.464
df	3	3	3
Asymp. Sig.	.000	.000	.001

Table 20c. Experiment III Phase A2 Home Cage Activity Kruskal-Wallis

Kruskal-Wallis Test	Housing Condition	N	Mean Rank
Number Moving	Non-Enriched Male	16	31.19
	Non-Enriched Female	16	35.00
	Super-Enriched Male	16	31.19
	Super-Enriched Female	15	30.53
	Total	63	
Amount Activity	Non-Enriched Male	16	20.38
	Non-Enriched Female	16	19.38
	Super-Enriched Male	16	39.56
	Super-Enriched Female	15	49.80
	Total	63	
Level Activity	Non-Enriched Male	16	22.63
	Non-Enriched Female	16	16.75
	Super-Enriched Male	16	39.97
	Super-Enriched Female	15	49.77
	Total	63	
Grouping Variable: Housing Condition	**Number Moving**	**Activity Amount**	**Activity Level**
Chi-Square	2.267	33.625	34.558
df	3	3	3
Asymp. Sig.	.519	.000	.000

Table 21a. Experiment III Open Field Activity Between Subjects MANOVA

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Power
Sex	HACTV1	87300.334	1	87300.334	.013	.911	.000	.051
	VACTV1	5114.839	1	5114.839	.132	.718	.003	.065
	HACTV2	40154044.512	1	40154044.512	2.704	.107	.059	.362
	VACTV2	69409.600	1	69409.600	.511	.479	.012	.108
	HACTV3	27727344.089	1	27727344.089	1.745	.194	.039	.252
	VACTV3	960070.251	1	960070.251	4.083	.050	.087	.506
Housing	HACTV1	1675547208.890	1	1675547208.890	242.947	.000	.850	1.000
	VACTV1	4397587.691	1	4397587.691	113.804	.000	.726	1.000
	HACTV2	1443860935.890	1	1443860935.890	97.225	.000	.693	1.000
	VACTV2	6167397.778	1	6167397.778	45.398	.000	.514	1.000
	HACTV3	1397771021.111	1	1397771021.111	87.960	.000	.672	1.000
	VACTV3	16883309.051	1	16883309.051	71.806	.000	.625	1.000
Sex * Housing	HACTV1	338066.556	1	338066.556	.049	.826	.001	.055
	VACTV1	53004.972	1	53004.972	1.372	.248	.031	.209
	HACTV2	4248568.956	1	4248568.956	.286	.595	.007	.082
	VACTV2	219699.600	1	219699.600	1.617	.210	.036	.237
	HACTV3	5833201.422	1	5833201.422	.367	.548	.008	.091
	VACTV3	1208757.614	1	1208757.614	5.141	.028	.107	.601
Error	HACTV1	296561000.136	43	6896767.445				
	VACTV1	1661598.742	43	38641.831				
	HACTV2	638578040.303	43	14850652.100				
	VACTV2	5841606.826	43	135851.322				
	HACTV3	683309874.326	43	15890927.310				
	VACTV3	10110342.886	43	235124.253				
	HACTV3	2121217145.106	46					
	VACTV3	28762204.213	46					

Table 21b. Experiment III Open Field Activity Between Subjects Tukey HSD Post-Hoc Comparisons

Variable	(I) housing	(J) housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
HACTV1	Non-Enriched Males	Non-Enriched Females	83.485	1096.225	1.000	-2846.091	3013.061
		Super-Enriched Males	12119.735(*)	1096.225	.000	9190.159	15049.311
		Super-Enriched Females	11863.735(*)	1096.225	.000	8934.159	14793.311
	Non-Enriched Females	Non-Enriched Males	-83.485	1096.225	1.000	-3013.061	2846.091
		Super-Enriched Males	12036.250(*)	1072.129	.000	9171.068	14901.432
		Super-Enriched Females	11780.250(*)	1072.129	.000	8915.068	14645.432
	Super-Enriched Males	Non-Enriched Males	-12119.735(*)	1096.225	.000	-15049.311	-9190.159
		Non-Enriched Females	-12036.250(*)	1072.129	.000	-14901.432	-9171.068
		Super-Enriched Females	-256.000	1072.129	.995	-3121.182	2609.182
	Super-Enriched Females	Non-Enriched Males	-11863.735(*)	1096.225	.000	-14793.311	-8934.159
		Non-Enriched Females	-11780.250(*)	1072.129	.000	-14645.432	-8915.068
		Super-Enriched Males	256.000	1072.129	.995	-2609.182	3121.182
VACTV1	Non-Enriched Males	Non-Enriched Females	-88.091	82.055	.707	-307.377	131.195
		Super-Enriched Males	544.992(*)	82.055	.000	325.706	764.278
		Super-Enriched Females	591.326(*)	82.055	.000	372.040	810.612
	Non-Enriched Females	Non-Enriched Males	88.091	82.055	.707	-131.195	307.377
		Super-Enriched Males	633.083(*)	80.251	.000	418.617	847.549
		Super-Enriched Females	679.417(*)	80.251	.000	464.951	893.883
	Super-Enriched Males	Non-Enriched Males	-544.992(*)	82.055	.000	-764.278	-325.706
		Non-Enriched Females	-633.083(*)	80.251	.000	-847.549	-418.617
		Super-Enriched Females	46.333	80.251	.938	-168.133	260.799
	Super-Enriched Females	Non-Enriched Males	-591.326(*)	82.055	.000	-810.612	-372.040
		Non-Enriched Females	-679.417(*)	80.251	.000	-893.883	-464.951
		Super-Enriched Males	-46.333	80.251	.938	-260.799	168.133
HACTV2	Non-Enriched Males	Non-Enriched Females	-1248.182	1608.606	.865	-5547.057	3050.693
		Super-Enriched Males	11694.818(*)	1608.606	.000	7395.943	15993.693
		Super-Enriched Females	9243.152(*)	1608.606	.000	4944.277	13542.026
	Non-Enriched Females	Non-Enriched Males	1248.182	1608.606	.865	-3050.693	5547.057
		Super-Enriched Males	12943.000(*)	1573.247	.000	8738.618	17147.382
		Super-Enriched Females	10491.333(*)	1573.247	.000	6286.951	14695.716
	Super-Enriched Males	Non-Enriched Males	-11694.818(*)	1608.606	.000	-15993.693	-7395.943
		Non-Enriched Females	-12943.000(*)	1573.247	.000	-17147.382	-8738.618
		Super-Enriched Females	-2451.667	1573.247	.413	-6656.049	1752.716
	Super-Enriched Females	Non-Enriched Males	-9243.152(*)	1608.606	.000	-13542.026	-4944.277
		Non-Enriched Females	-10491.333(*)	1573.247	.000	-14695.716	-6286.951
		Super-Enriched Males	2451.667	1573.247	.413	-1752.716	6656.049

Table 21b. Experiment III Open Field Activity Between Subjects Tukey HSD Post-Hoc Comparisons Continued

Variable	(I) housing	(J) housing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
VACTV2	Non-Enriched Males	Non-Enriched Females	59.924	153.8540	.980	-351.239	471.087
		Super-Enriched Males	861.841(*)	153.8540	.000	450.678	1273.004
		Super-Enriched Females	648.091(*)	153.8540	.001	236.928	1059.254
	Non-Enriched Females	Non-Enriched Males	-59.924	153.8540	.980	-471.087	351.239
		Super-Enriched Males	801.917(*)	150.4722	.000	399.791	1204.042
		Super-Enriched Females	588.167(*)	150.4722	.002	186.041	990.292
	Super-Enriched Males	Non-Enriched Males	-861.841(*)	153.8540	.000	-1273.004	-450.678
		Non-Enriched Females	-801.917(*)	150.4722	.000	-1204.042	-399.791
		Super-Enriched Females	-213.750	150.4722	.494	-615.875	188.375
	Super-Enriched Females	Non-Enriched Males	-648.091(*)	153.8540	.001	-1059.254	-236.928
		Non-Enriched Females	-588.167(*)	150.4722	.002	-990.292	-186.041
		Super-Enriched Males	213.750	150.4722	.494	-188.375	615.875
HACTV3	Non-Enriched Males	Non-Enriched Females	-832.159	1663.9933	.959	-5279.052	3614.734
		Super-Enriched Males	11619.674(*)	1663.9933	.000	7172.782	16066.567
		Super-Enriched Females	9377.341(*)	1663.9933	.000	4930.448	13824.234
	Non-Enriched Females	Non-Enriched Males	832.159	1663.9933	.959	-3614.734	5279.052
		Super-Enriched Males	12451.833(*)	1627.4176	.000	8102.687	16800.980
		Super-Enriched Females	10209.500(*)	1627.4176	.000	5860.353	14558.647
	Super-Enriched Males	Non-Enriched Males	-11619.674(*)	1663.9933	.000	-16066.567	-7172.782
		Non-Enriched Females	-12451.833(*)	1627.4176	.000	-16800.980	-8102.687
		Super-Enriched Females	-2242.333	1627.4176	.520	-6591.480	2106.813
	Super-Enriched Females	Non-Enriched Males	-9377.341(*)	1663.9933	.000	-13824.234	-4930.448
		Non-Enriched Females	-10209.500(*)	1627.4176	.000	-14558.647	-5860.353
		Super-Enriched Males	2242.333	1627.4176	.520	-2106.813	6591.480
VACTV3	Non-Enriched Males	Non-Enriched Females	607.015(*)	202.4071	.022	66.098	1147.932
		Super-Enriched Males	1520.515(*)	202.4071	.000	979.598	2061.432
		Super-Enriched Females	1485.598(*)	202.4071	.000	944.681	2026.516
	Non-Enriched Females	Non-Enriched Males	-607.015(*)	202.4071	.022	-1147.932	-66.098
		Super-Enriched Males	913.500(*)	197.9580	.000	384.473	1442.527
		Super-Enriched Females	878.583(*)	197.9580	.000	349.556	1407.611
	Super-Enriched Males	Non-Enriched Males	-1520.515(*)	202.4071	.000	-2061.432	-979.598
		Non-Enriched Females	-913.500(*)	197.9580	.000	-1442.527	-384.473
		Super-Enriched Females	-34.917	197.9580	.998	-563.944	494.111
	Super-Enriched Females	Non-Enriched Males	-1485.598(*)	202.4071	.000	-2026.516	-944.681
		Non-Enriched Females	-878.583(*)	197.9580	.000	-1407.611	-349.556
		Super-Enriched Males	34.917	197.9580	.998	-494.111	563.944

Table 21c. Experiment III Open Field Activity Tests of Between-Subjects Effects

Variables	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	HACTV1	87300.334	1	87300.334	.013	.911	.000	.051
	VACTV1	5114.839	1	5114.839	.132	.718	.003	.065
	HACTV2	40154044.512	1	40154044.512	2.704	.107	.059	.362
	VACTV2	69409.600	1	69409.600	.511	.479	.012	.108
	HACTV3	27727344.089	1	27727344.089	1.745	.194	.039	.252
	VACTV3	960070.251	1	960070.251	4.083	.050	.087	.506
Housing	HACTV1	1675547208.890	1	1675547208.890	242.947	.000	.850	1.000
	VACTV1	4397587.691	1	4397587.691	113.804	.000	.726	1.000
	HACTV2	1443860935.890	1	1443860935.890	97.225	.000	.693	1.000
	VACTV2	6167397.778	1	6167397.778	45.398	.000	.514	1.000
	HACTV3	1397771021.111	1	1397771021.111	87.960	.000	.672	1.000
	VACTV3	16883309.051	1	16883309.051	71.806	.000	.625	1.000
Sex * Housing	HACTV1	338066.556	1	338066.556	.049	.826	.001	.055
	VACTV1	53004.972	1	53004.972	1.372	.248	.031	.209
	HACTV2	4248568.956	1	4248568.956	.286	.595	.007	.082
	VACTV2	219699.600	1	219699.600	1.617	.210	.036	.237
	HACTV3	5833201.422	1	5833201.422	.367	.548	.008	.091
	VACTV3	1208757.614	1	1208757.614	5.141	.028	.107	.601
Error	HACTV1	296561000.136	43	6896767.445				
	VACTV1	1661598.742	43	38641.831				
	HACTV2	638578040.303	43	14850652.100				
	VACTV2	5841606.826	43	135851.322				
	HACTV3	683309874.326	43	15890927.310				
	VACTV3	10110342.886	43	235124.253				

Table 21d. Experiment III OF Activity Within-Subjects Tests of Effects

Variable	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
OF 1 HACT Time	179062982.355	11	16278452.941	78.060	.000	.645	1.000
OF 1 VACT Time	835388.752	11	75944.432	58.025	.000	.574	1.000
OF 2 HACT Time	198650293.636	11	18059117.603	65.474	.000	.604	1.000
OF 2 VACT Time	1645486.979	11	149589.725	53.879	.000	.556	1.000
OF 3 HACT Time	148086744.719	11	13462431.338	63.529	.000	.596	1.000
OF 3 VACT Time	1624852.810	11	147713.892	37.862	.000	.468	1.000
OF 1 HACT Time * Sex	1869298.898	11	169936.263	.815	.625	.019	.459
OF 1 VACT Time * Sex	23444.134	11	2131.285	1.628	.088	.036	.821
OF 2 HACT Time * Sex	2526452.874	11	229677.534	.833	.607	.019	.469
OF 2 VACT Time * Sex	39339.538	11	3576.322	1.288	.228	.029	.700
OF 3 HACT Time * Sex	3497889.717	11	317989.974	1.501	.128	.034	.781
OF 3 VACT Time * Sex	54684.457	11	4971.314	1.274	.236	.029	.694
OF 1 HACT Time * Housing	49429383.276	11	4493580.298	21.548	.000	.334	1.000
OF 1 VACT Time * Housing	150164.234	11	13651.294	10.430	.000	.195	1.000
OF 2 HACT Time * Housing	25236562.674	11	2294232.970	8.318	.000	.162	1.000
OF 2 VACT Time * Housing	157702.146	11	14336.559	5.164	.000	.107	1.000
OF 3 HACT Time * Housing	14348871.532	11	1304442.867	6.156	.000	.125	1.000
OF 3 VACT Time * Housing	102818.176	11	9347.107	2.396	.007	.053	.956
OF 1 HACT Time * Sex * Housing	3597243.231	11	327022.112	1.568	.105	.035	.803
OF 1 VACT Time * Sex * Housing	22306.341	11	2027.849	1.549	.111	.035	.797
OF 2 HACT Time * Sex * Housing	3077974.740	11	279815.885	1.014	.432	.023	.569
OF 2 VACT Time * Sex * Housing	68168.816	11	6197.165	2.232	.012	.049	.939
OF 3 HACT Time * Sex * Housing	3830385.289	11	348216.844	1.643	.084	.037	.825
OF 3 VACT Time * Sex * Housing	74702.659	11	6791.151	1.741	.062	.039	.851
OF 1 HACT Error(Time)	98638596.502	473	208538.259				
OF 1 VACT Error(Time)	619075.908	473	1308.829				
OF 2 HACT Error(Time)	130464289.581	473	275823.022				
OF 2 VACT Error(Time)	1313229.878	473	2776.385				
OF 3 HACT Error(Time)	100232720.176	473	211908.499				
OF 3 VACT Error(Time)	1845333.552	473	3901.339				

Table 22. Mean Body Lengths For All Three Experiments

Experiment I (Males)	Mean Body Length	Standard Deviation
Non-Enriched	23.13	1.07
Enriched	23.73	0.66
Super-Enriched	23.14	1.02
Lengths were not significantly different		
Experiment II (Females)		
Non-Enriched	19.51	1.01
Enriched	19.09	0.57
Super-Enriched	19.30	0.60
Lengths were not significantly different		
Experiment III (Males & Females)		
Males Non-Enriched	23.77	0.81
Males Super-Enriched	23.78	1.09
Females Non-Enriched	20.19	1.17
Females Super-Enriched	19.4	0.71
Females were significantly shorter than males (F (1,44) = 203.647, p < 0.05) No significant effect for housing or housing X gender interaction		

Figures

Figure 1. Non-Enriched Housing

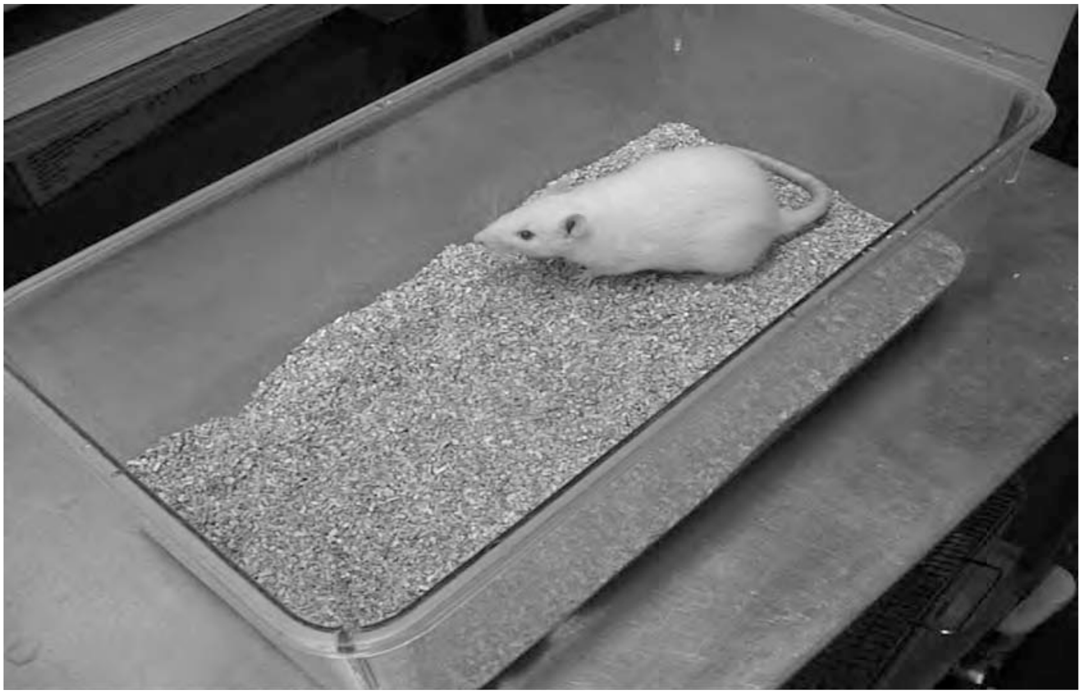


Figure 2. Enriched Housing

Figure 3. Super-Enriched Housing

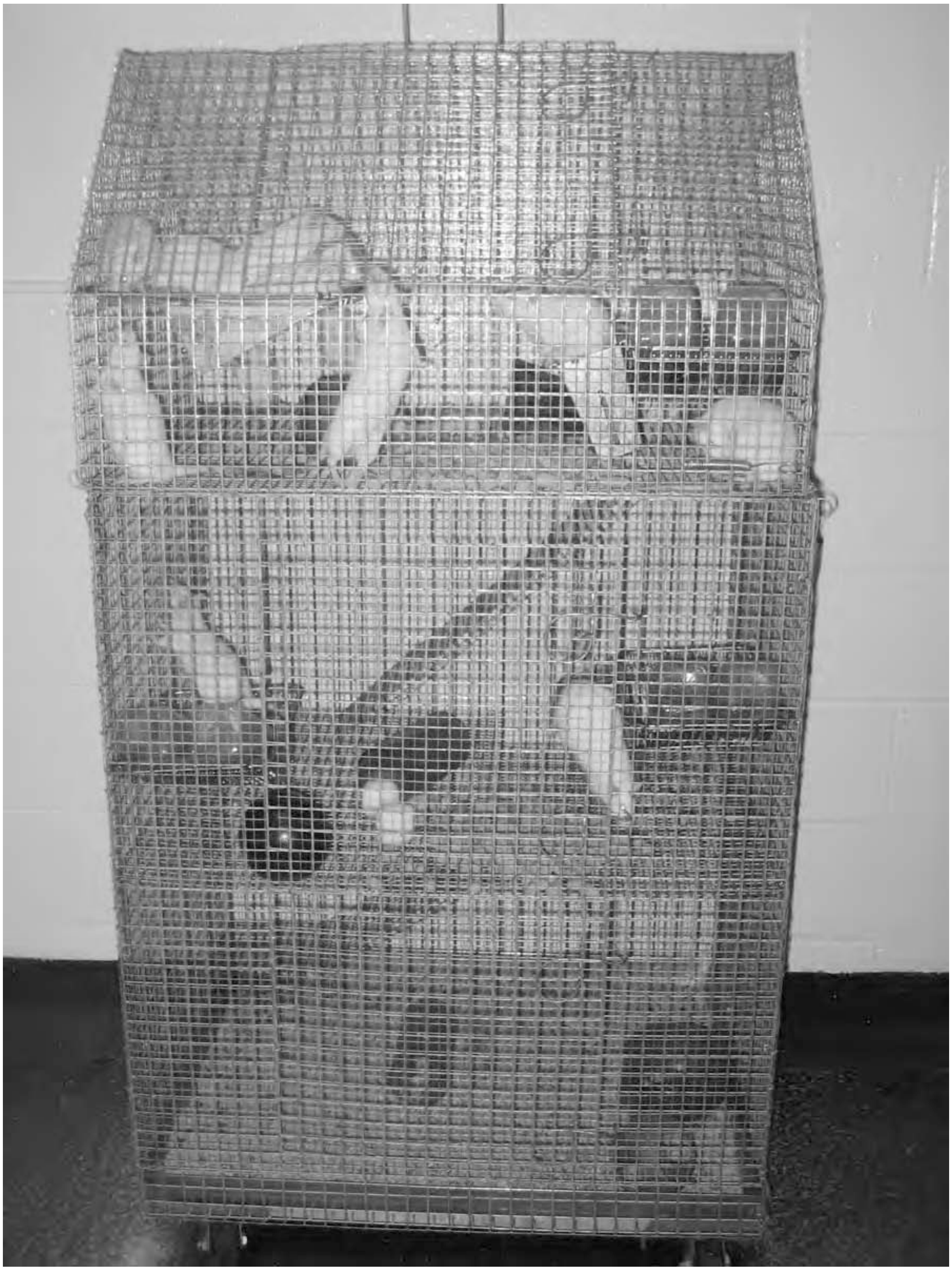


Figure 4. Individual Subject Activity Data Sheet

Date __________

Home Cage Activity (3 Minute Observations)

Subject:__________ **Condition:** __________ **Rater Initials:** _______

Circle a number between 1 and 7.

	1	2	3	4	5	6	7
Horizontal Movement	None	Almost No Activity	Low Activity	Some Activity	Moderate Activity	Intermittent High Activity	Continuous High Activity

	1	2	3	4	5	6	7
Vertical Movement	None	Almost No Activity	Low Activity	Some Activity	Moderate Activity	Intermittent High Activity	Continuous High Activity

	1	2	3	4	5	6	7
Level of In-Place Movement	None	Almost No Activity	Low Activity	Some Activity	Moderate Activity	Intermittent High Activity	Continuous High Activity

	1	2	3	4	5	6	7
Level Locomotor Movement	None	Almost No Activity	Low Activity	Some Activity	Moderate Activity	Intermittent High Activty	Continuous High Activity

	1	2	3	4	5	6	7
Center Time Movement	None	Almost No Center Activity	Low	Evenly Split	Moderate Activity	High Center Activity	All Center

Description/Comments:____

____

____

____

____

Figure 5. Group Activity Data Sheet

Date ___________

Time __________

Home Cage Activity (1 Minute Observations)

Condition: ___________ Rater Initials: ________ Experimental Day ________

Circle a number between 1 and 7.

Number of Animals Moving

1	2	3	4	5	6	7
None	1-2	3-4	5-6	7-8	9-10	11-12

Amount of Activity for Majority of Group Members

1	2	3	4	5	6	7
None	Almost No Activity	Low Activity	Some Activity	Moderate Activity	Intermittent High Activty	Continuous High Activity

Level of Activity

1	2	3	4	5	6	7
None	Almost No Effort	Low Effort	Some Effort	Moderate Effort	Intermittent High Effort	Continuous High Effort

Indicate the type of activity and the number of animals engaged in each type of activity:

w/Physical Object	Social Interaction	Combined P & S	Alone
_________________	_______________	______________	_______

Description/Comments:____

Figure 6. Open Field Chamber

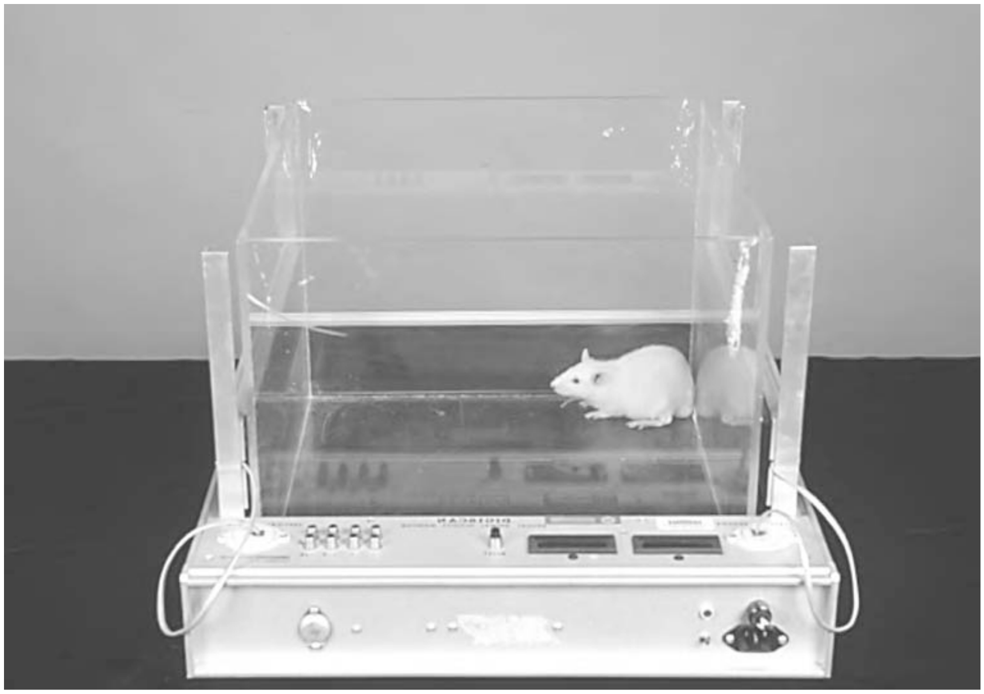


Figure 7a. Experiment I Body Weights (bar)

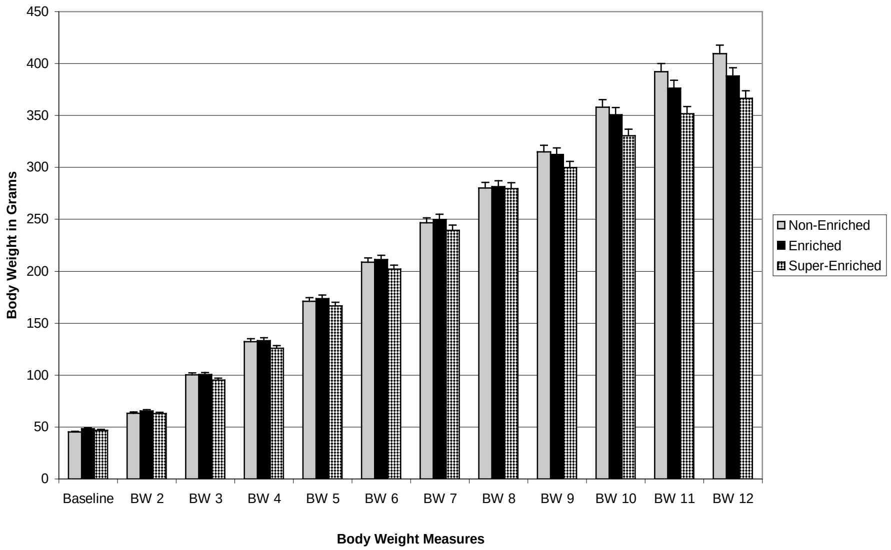


Figure 7b. Experiment I Body Weights (line)

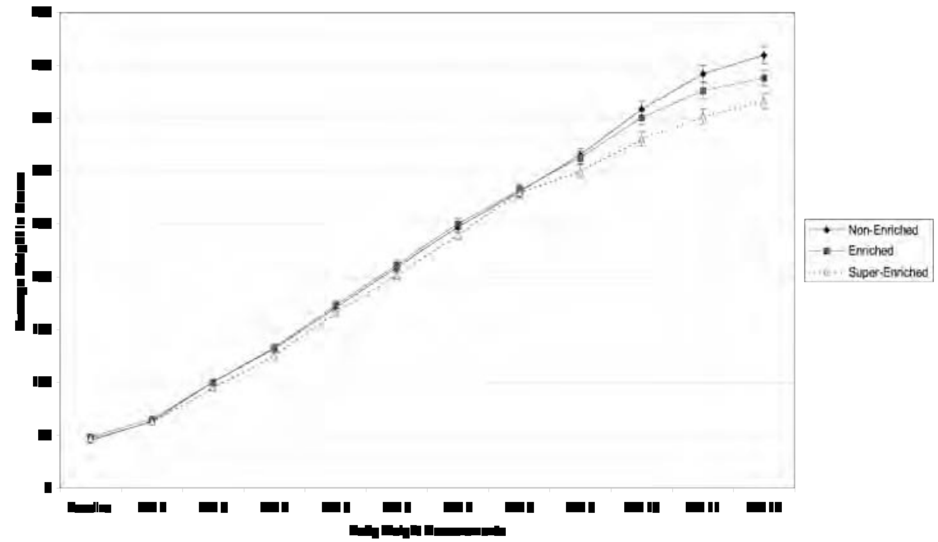


Figure 7c. Experiment I Body Weight by Phase

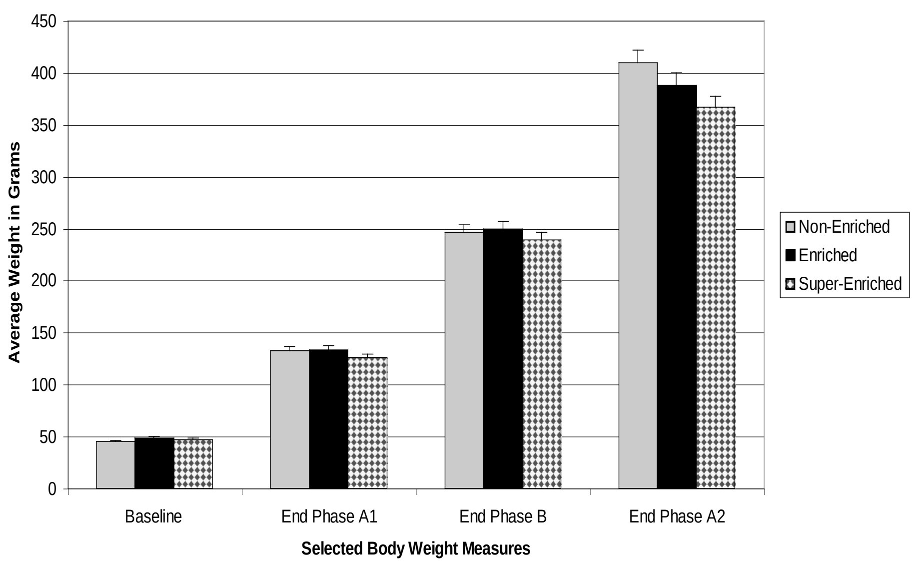


Figure 8. Experiment I Body Mass Index

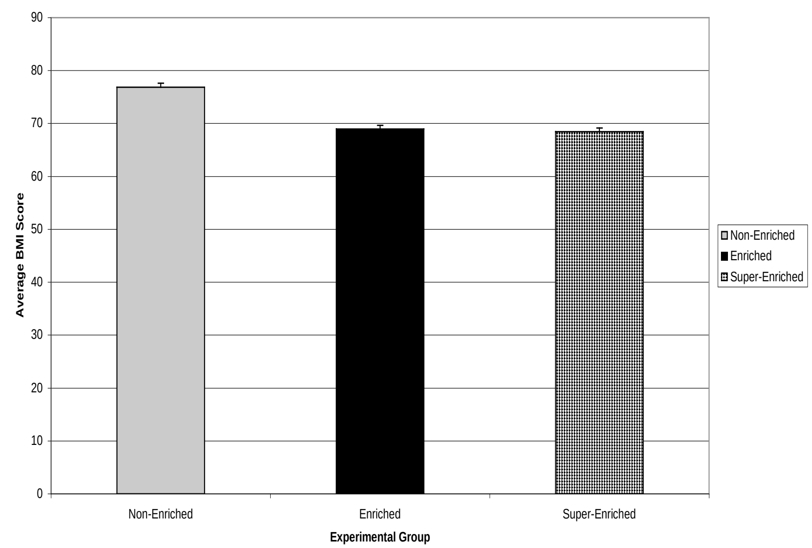


Figure 9. Experiment I Lee Index

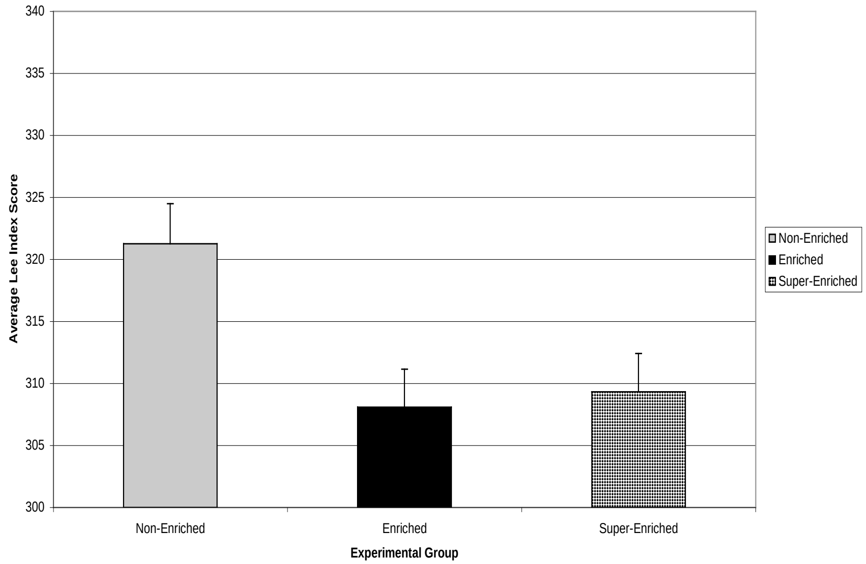


Figure 10a. Experiment I Average Number of Grams Consumed Daily by Experimental Phase

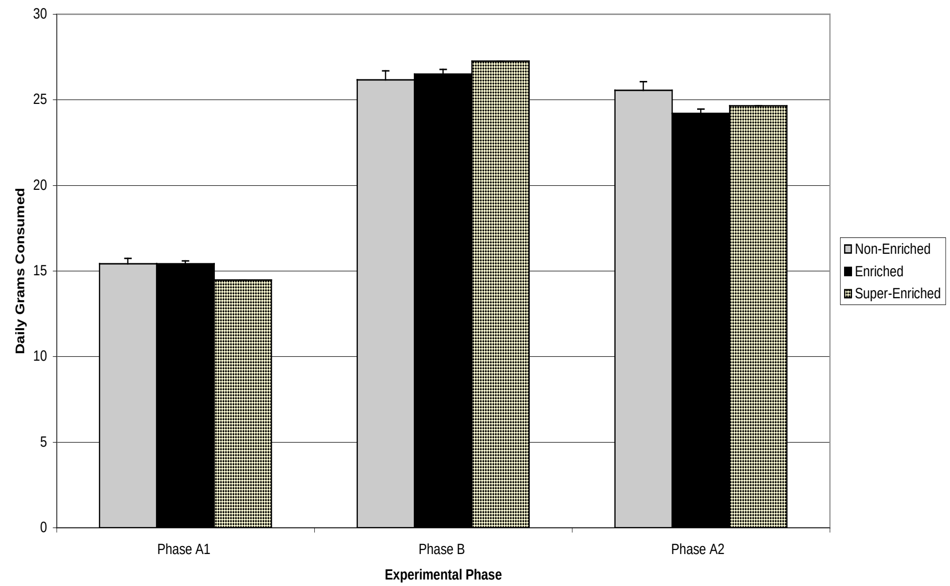


Figure 10b. Experiment I Average Number of Calories Consumed Daily by Experimental Phase

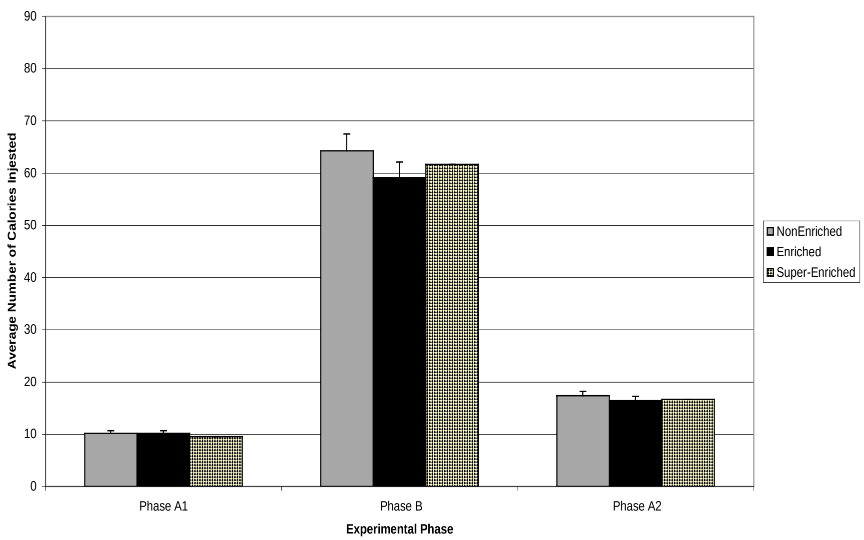


Figure 10c. Experiment I Phase B Average Grams Breakdown

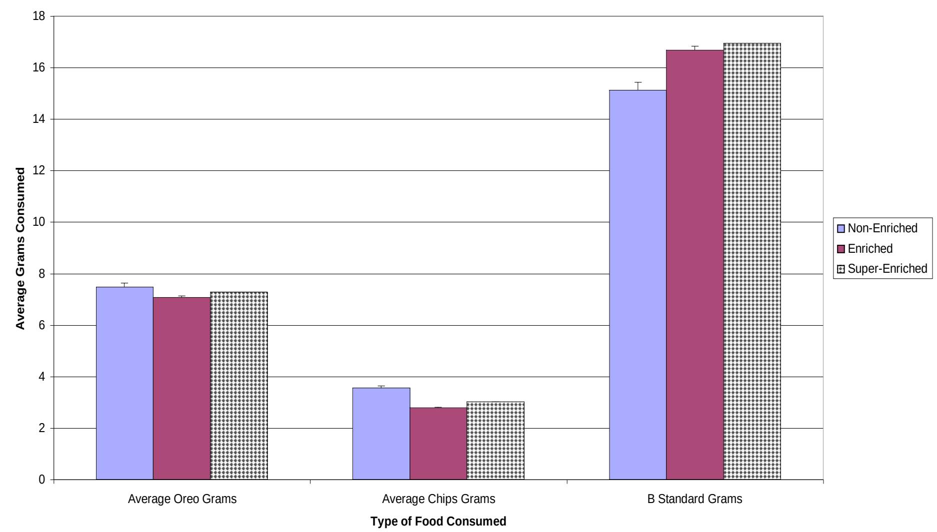


Figure 10d. Experiment I Phase B Average Calories Breakdown

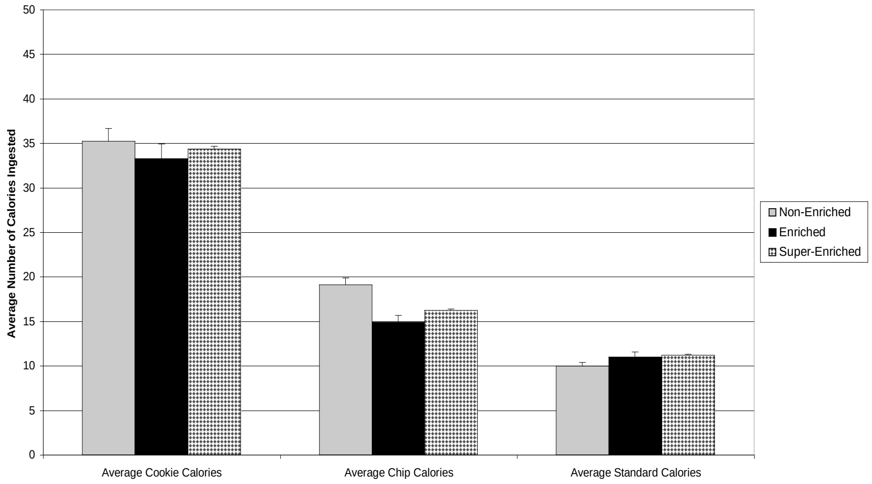


Figure11a. Experiment I Median Score for Number of Animals Moving by Experimental Phase

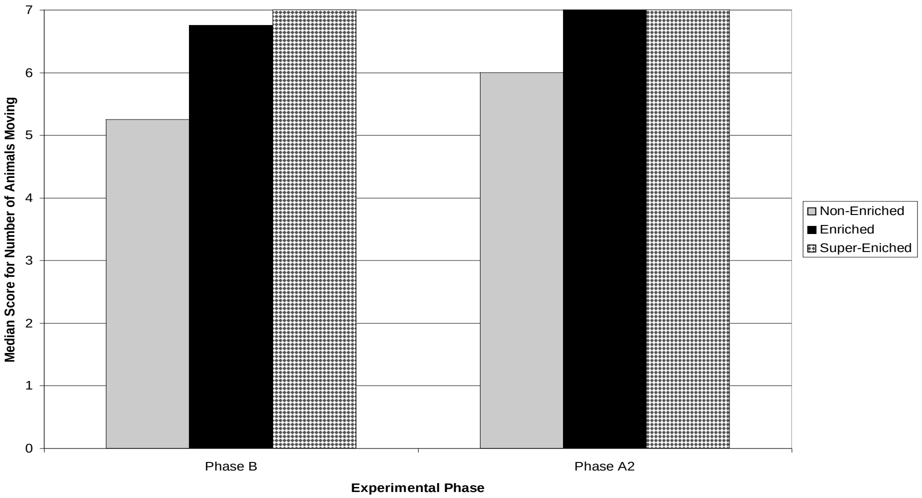


Figure 11b. Experiment I Median Score for Amount of Physical Activity

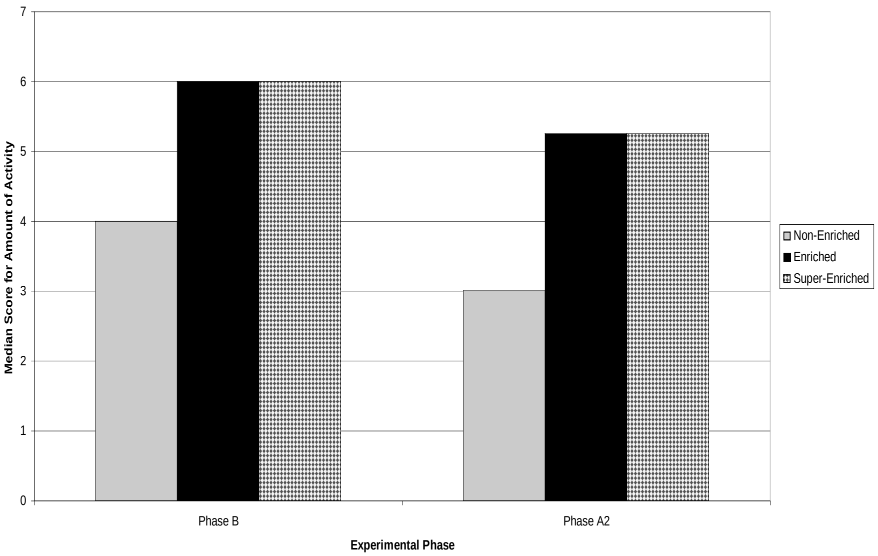


Figure 11c. Experiment I Median Score for Level of Physical Activity

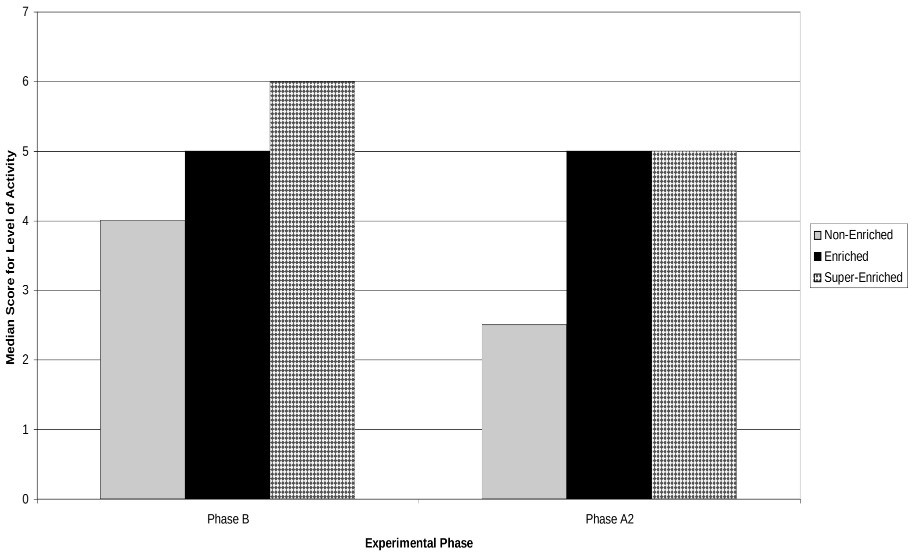


Figure 12a. Experiment I Horizontal Activity Separated by Experimental Phase

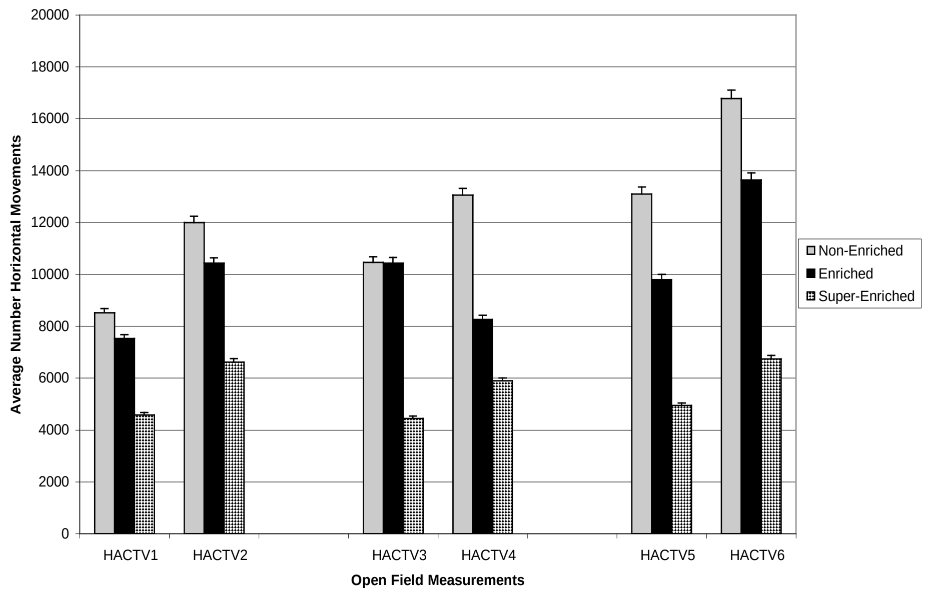


Figure 12b. Experiment I Vertical Activity Separated by Experimental Phase

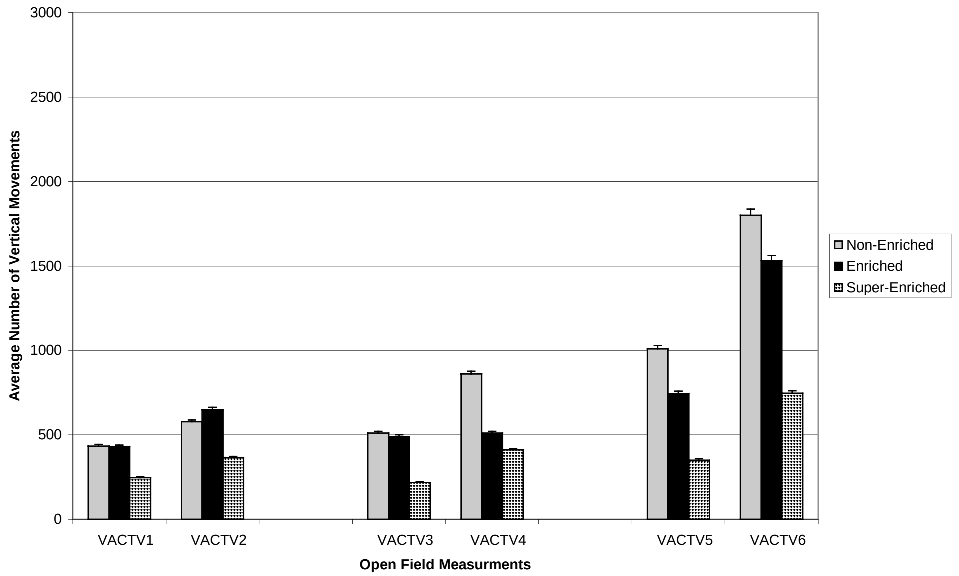


Figure 12c. Experiment I Average Horizontal Activity Separated by Experimental Phase

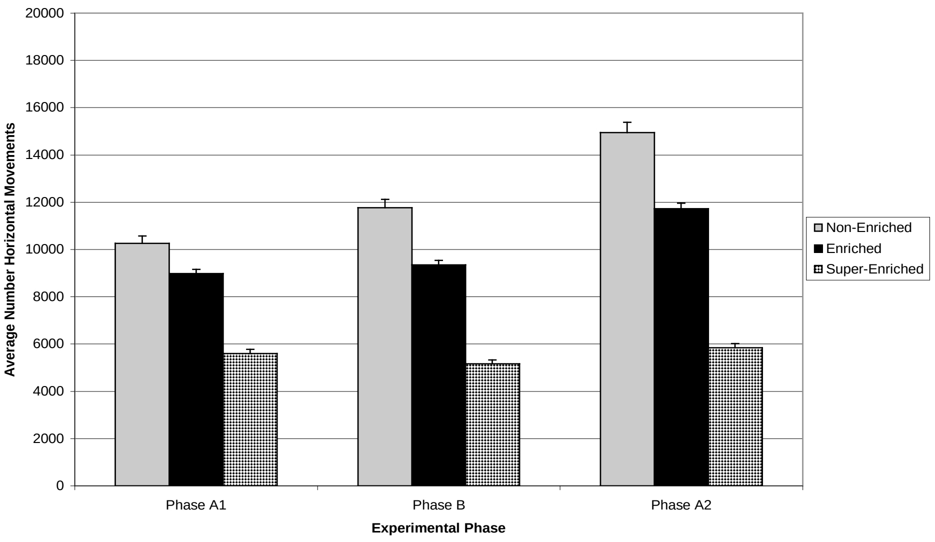


Figure 12d. Experiment I Average Vertical Activity Separated by Experimental Phase

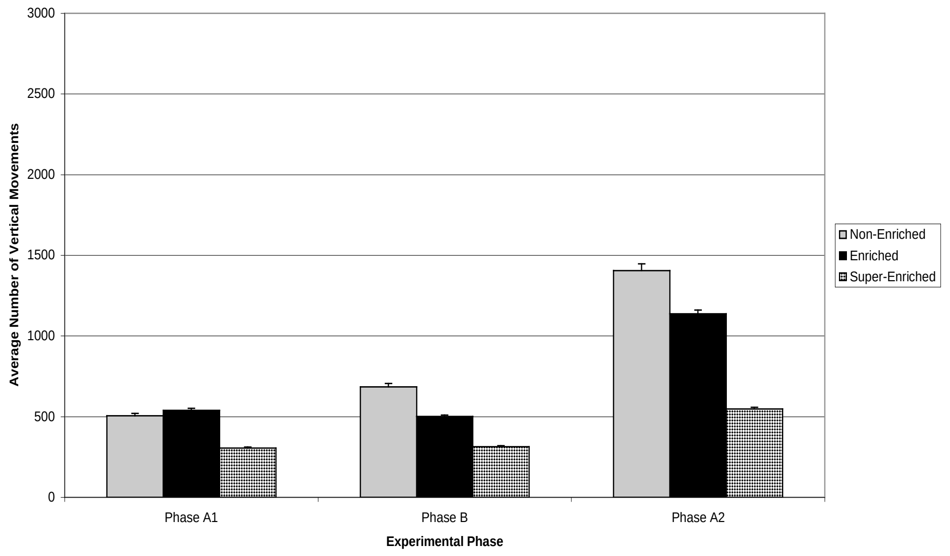


Figure12e. Experiment I Averaged Within Session Horizontal Activity From All OF Measurements (HACT from all 6 OF trials averaged

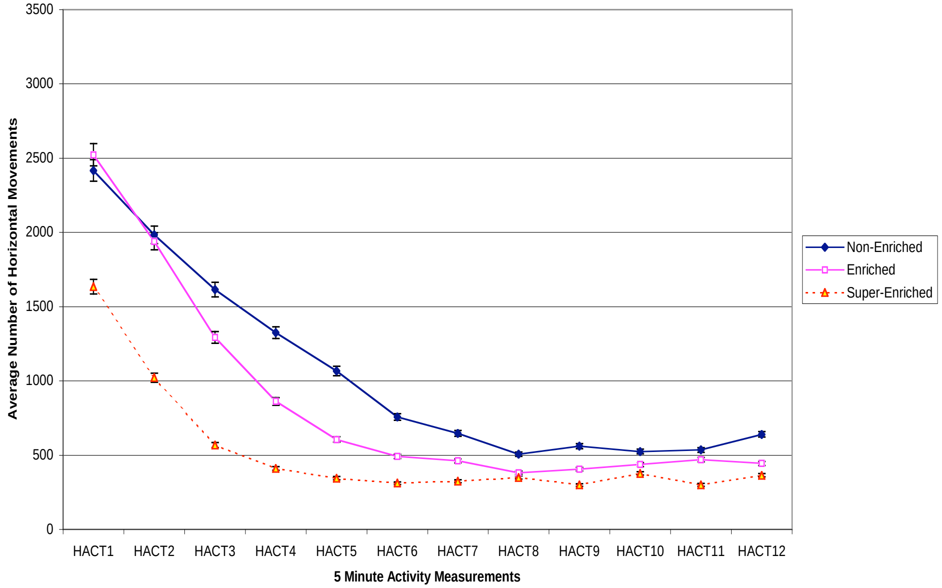


Figure12f. Experiment I Averaged Within Session Vertical Activity From All OF Measurements (VACT from all 6 OF trials averaged together)

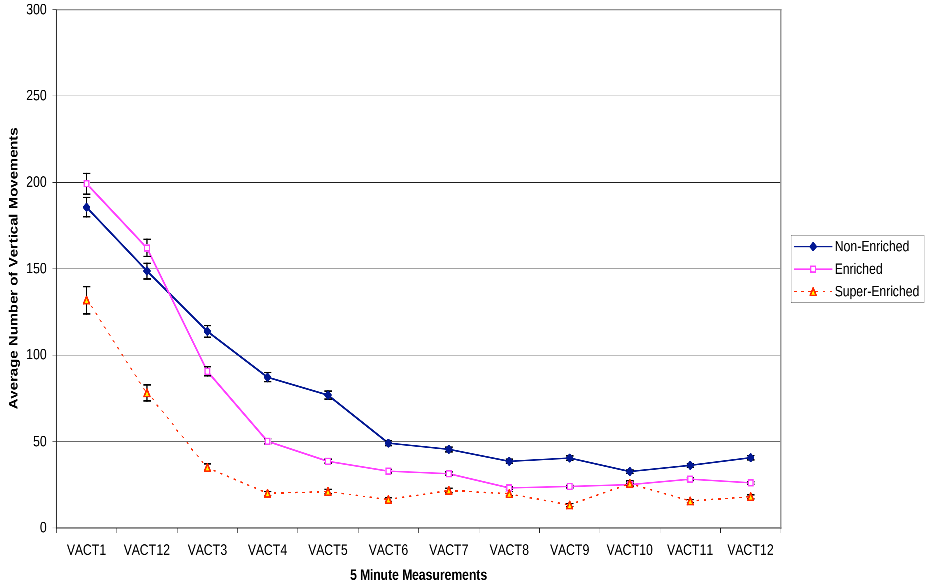


Figure 13a. Experiment II Body Weights (bar)

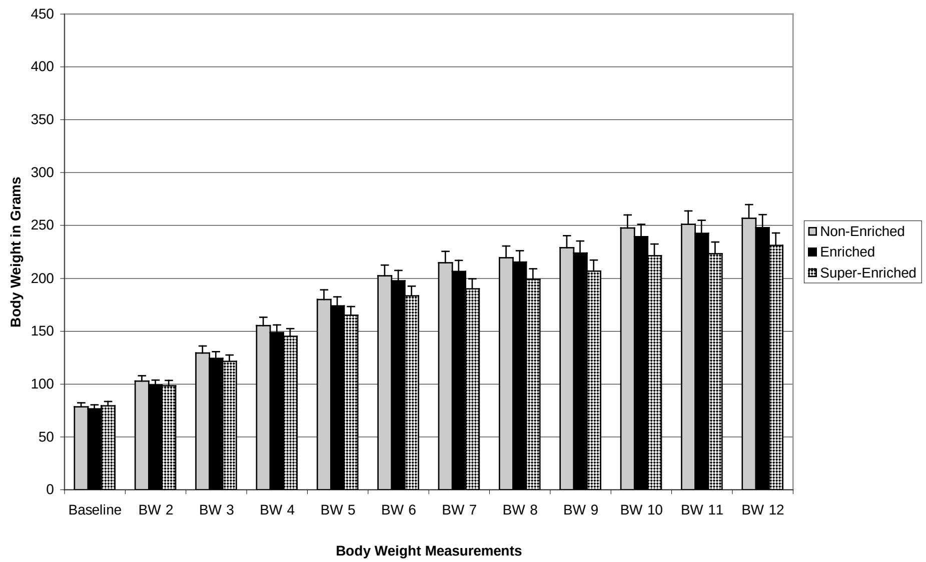


Figure 13b. Experiment II Body Weights (line)

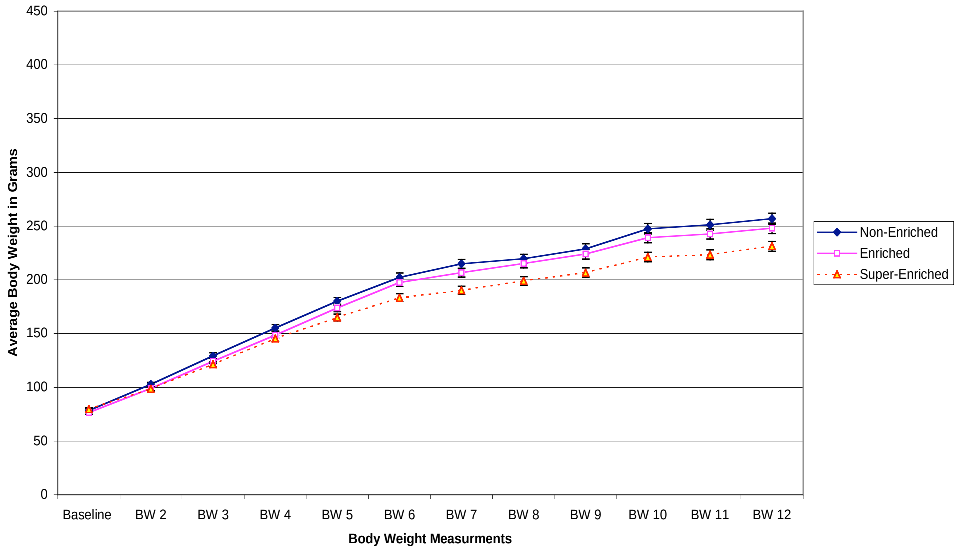


Figure13b. Experiment II Body Weight by Experimental Phase (bar)

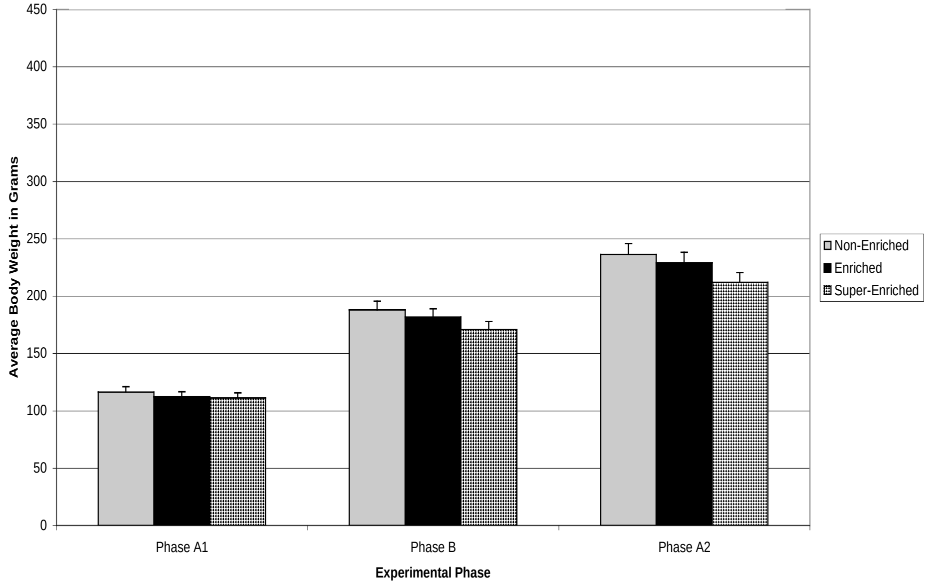


Figure14. Experiment II Body Mass Index

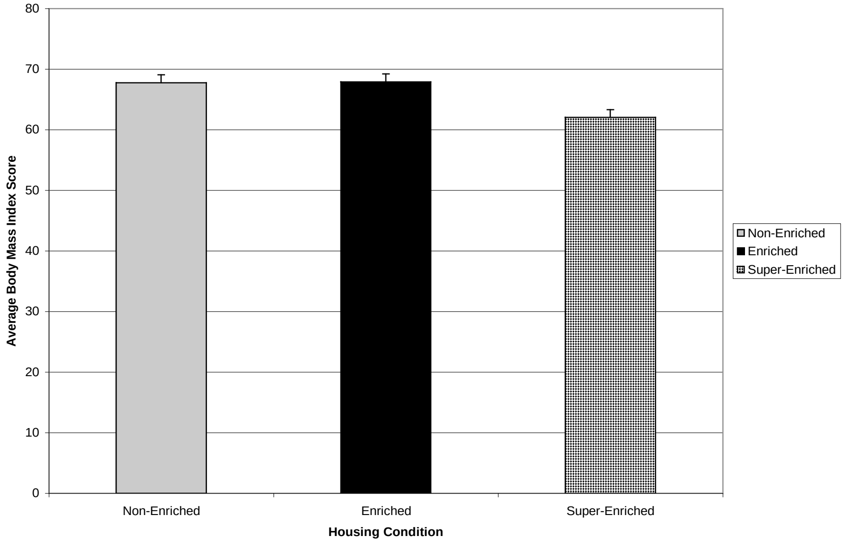


Figure15. Experiment II Lee Index

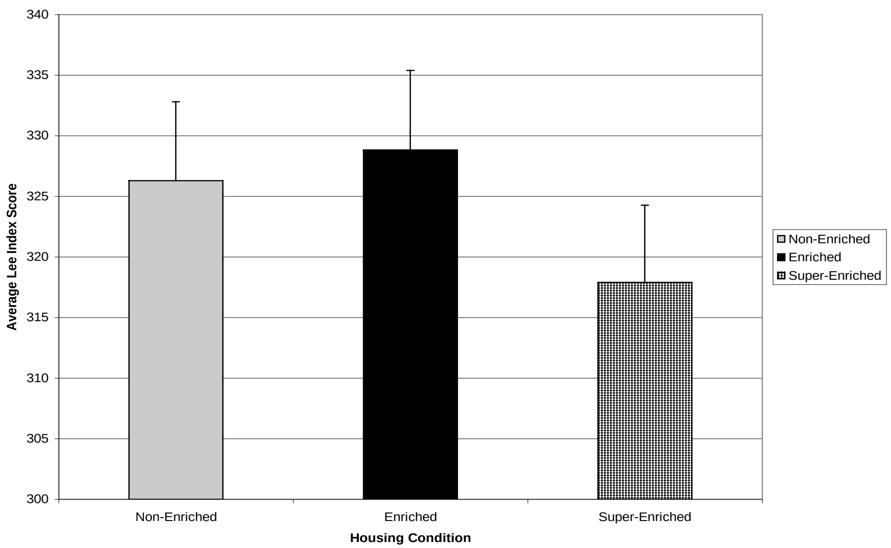


Figure16a. Experiment II Average Number of Grams Consumed Daily by Experimental Phase

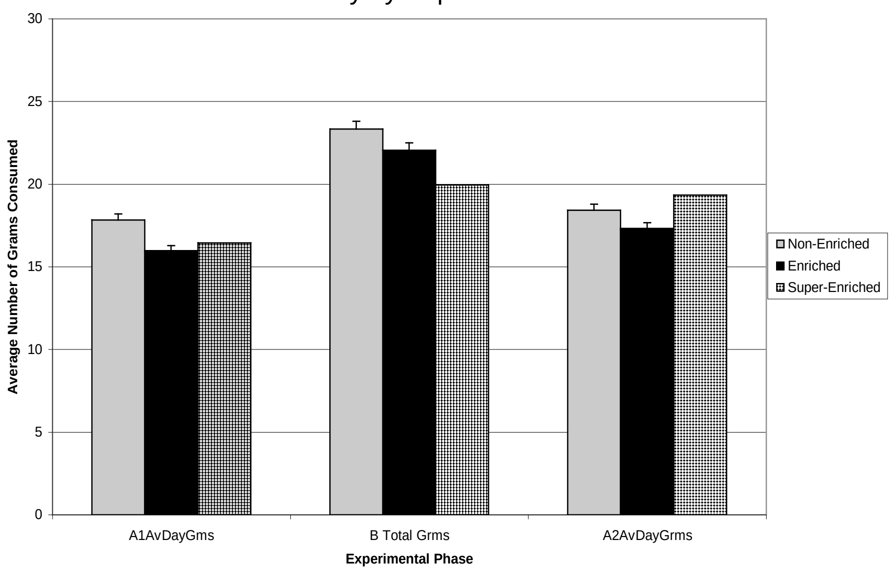


Figure16b. Experiment II Average Number of Calories Consumed Daily by Experimental Phase

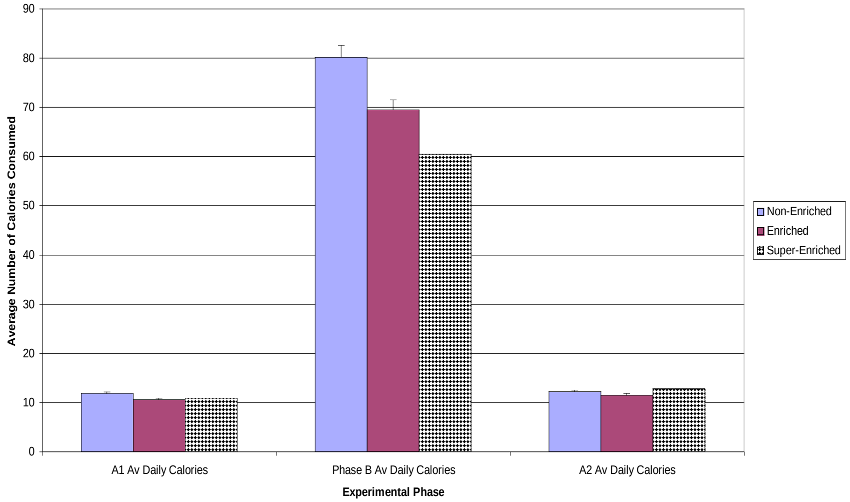


Figure16c. Experiment II Phase B Average Grams Breakdown

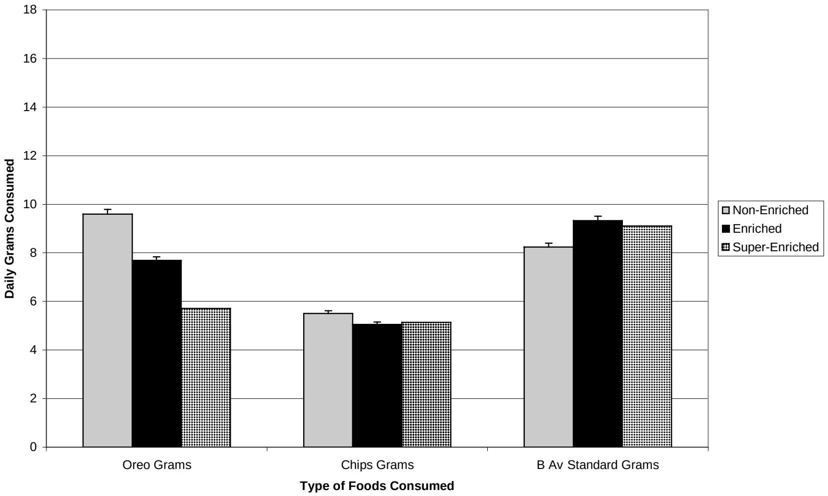


Figure 16d. Experiment II Phase B Average Calories Breakdown

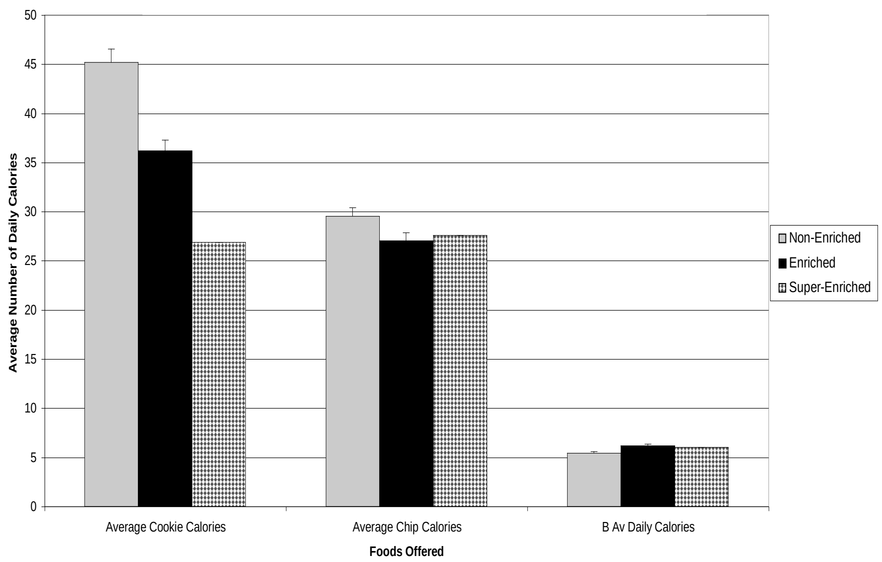


Figure17a. Experiment II Median Score for Number of Animals Moving by Experimental Phase

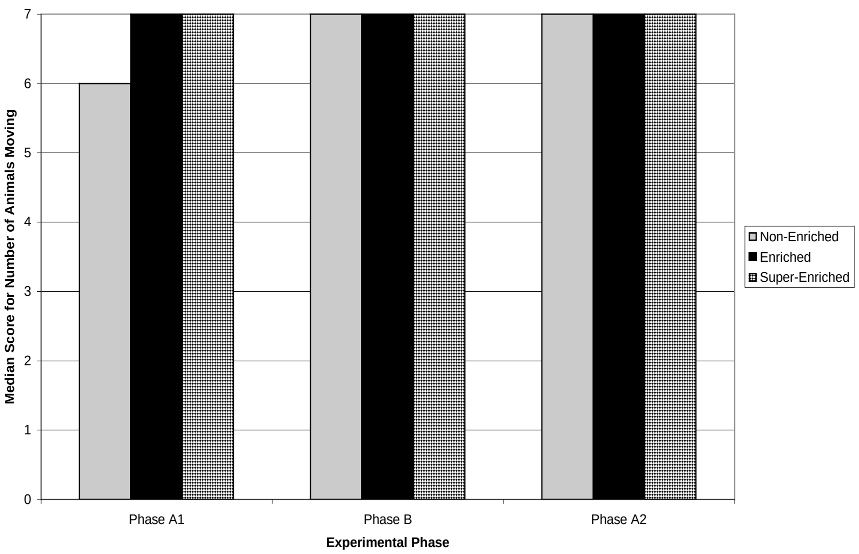


Figure 17b. Experiment II Median Score for Amount of Physical Activity by Experimental Phase

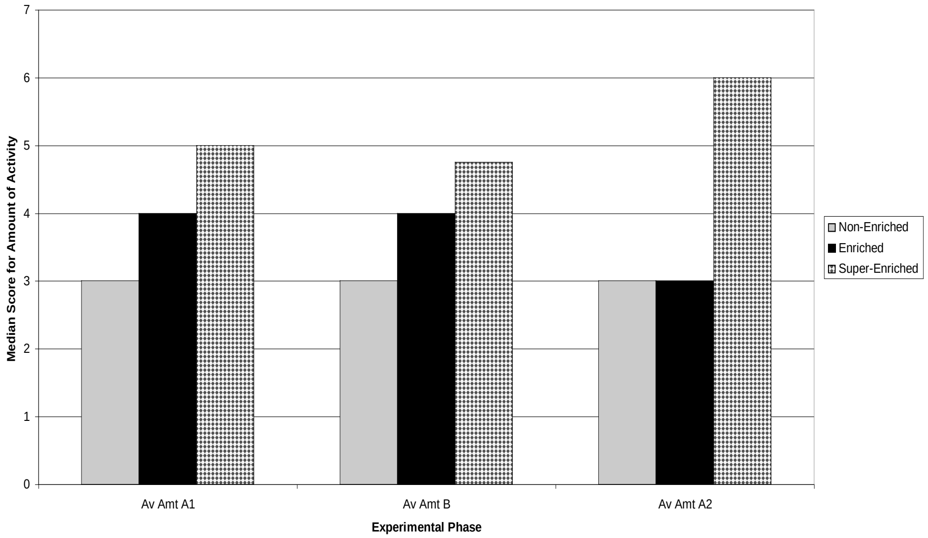


Figure 17c. Experiment II Median Score for Level of Physical Activity by Experimental Phase

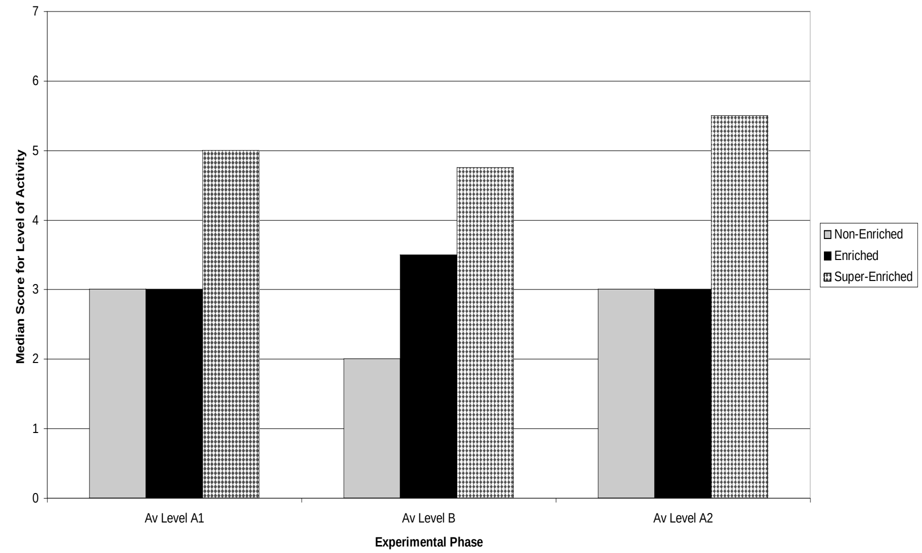


Figure 18a. Experiment II Horizontal Activity Separated by Experimental Phase

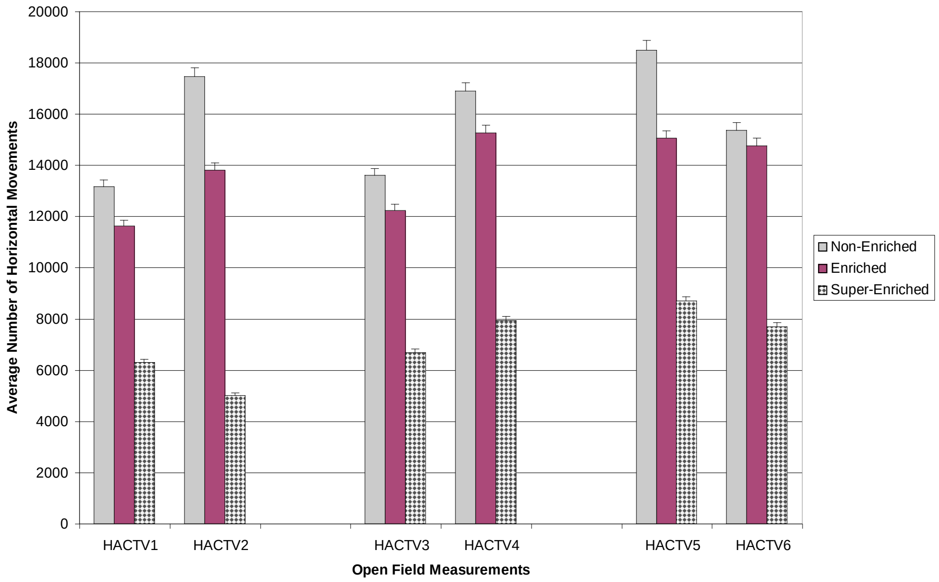


Figure 18b. Experiment II Averaged Horizontal Activity Separated by Experimental Phase

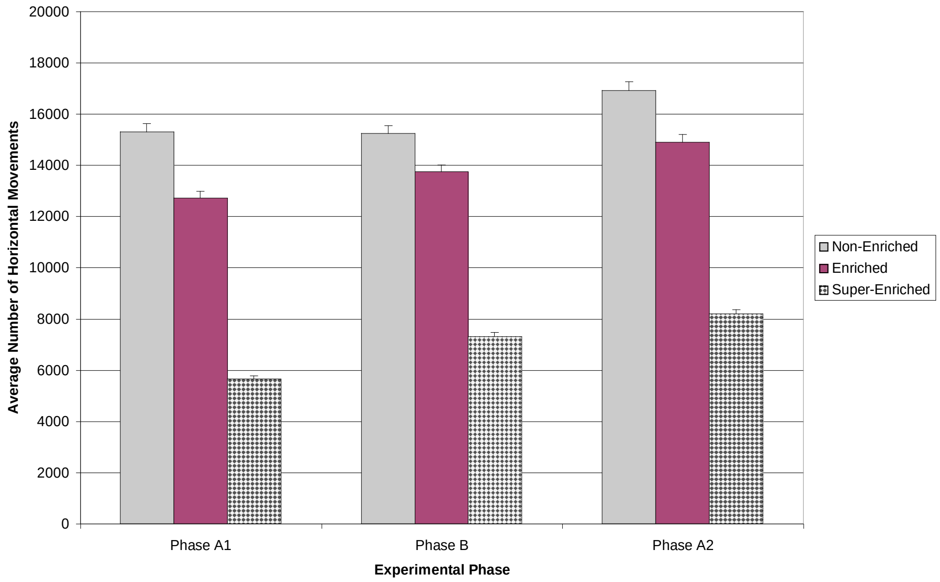


Figure 18c. Experiment II Vertical Activity Separated by Experimental Phase

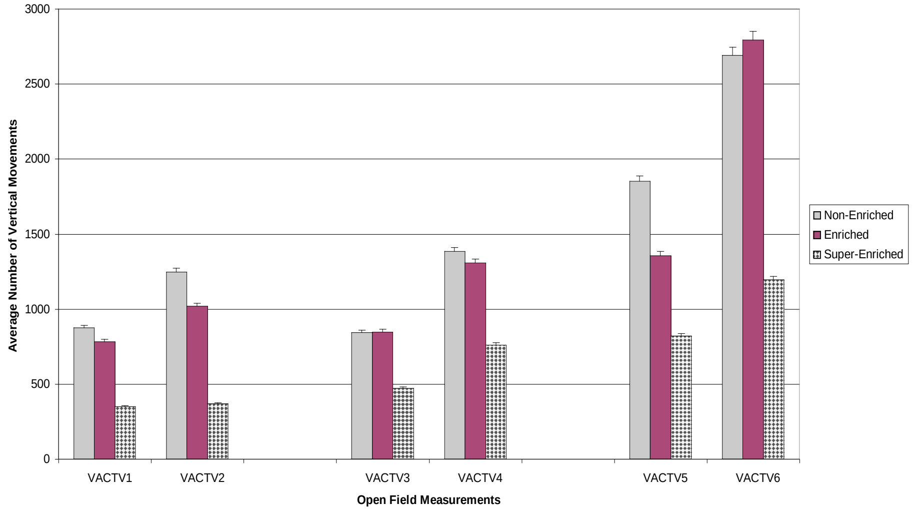


Figure 18d. Experiment II Averaged Vertical Activity Separated by Experimental Phase

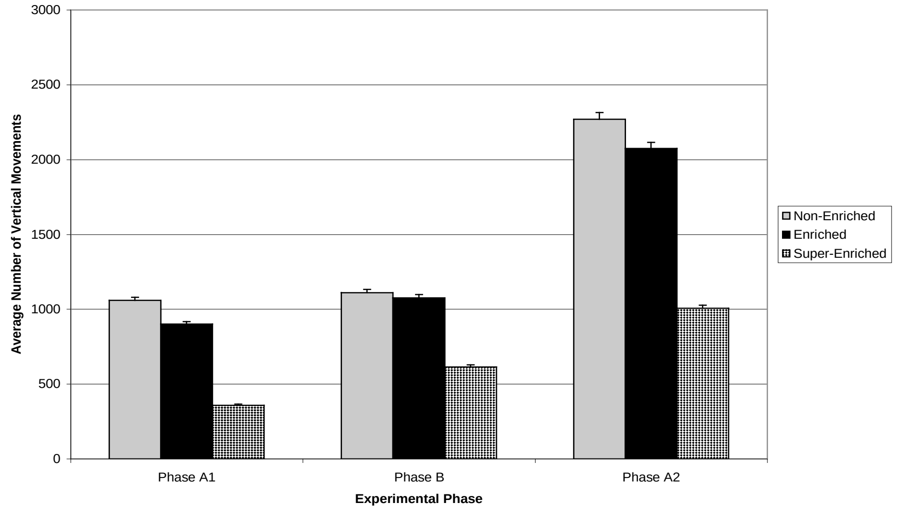


Figure 18e. Experiment II Averaged Within Session Horizontal Activity From All OF Measurements (HACT from all 6 OF trials averaged together)

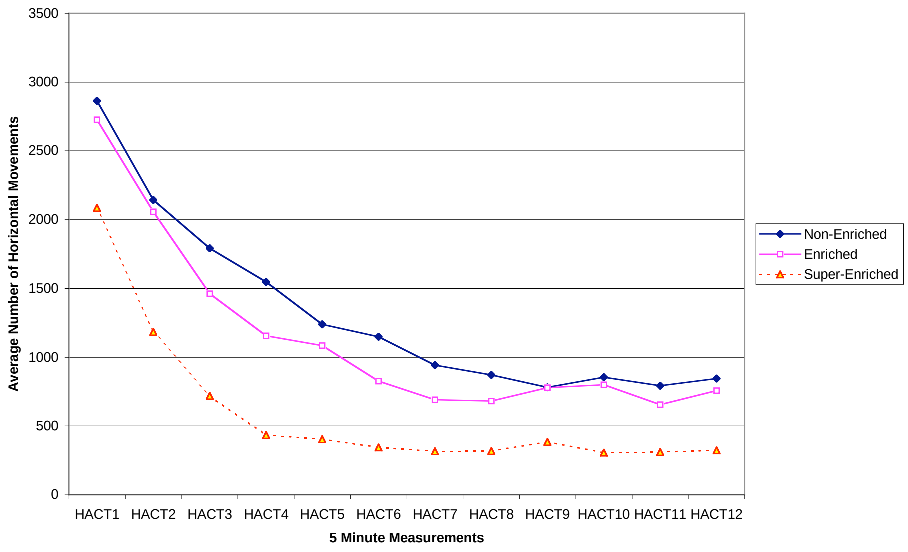


Figure 18f. Experiment II Averaged Within Session Vertical Activity From All OF Measurements (VACT from all 6 OF trials averaged together)

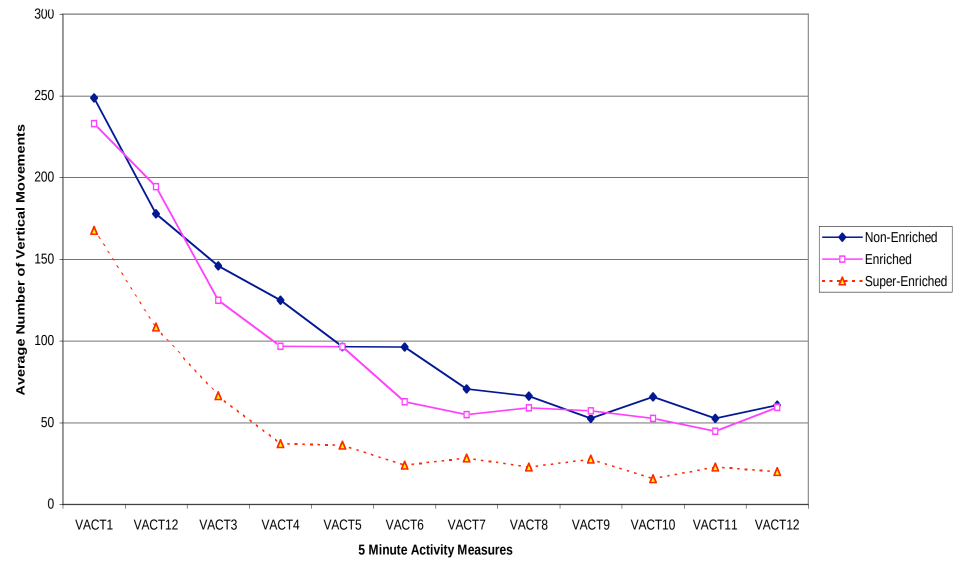


Figure 19a. Experiment III Body Weight (bar)

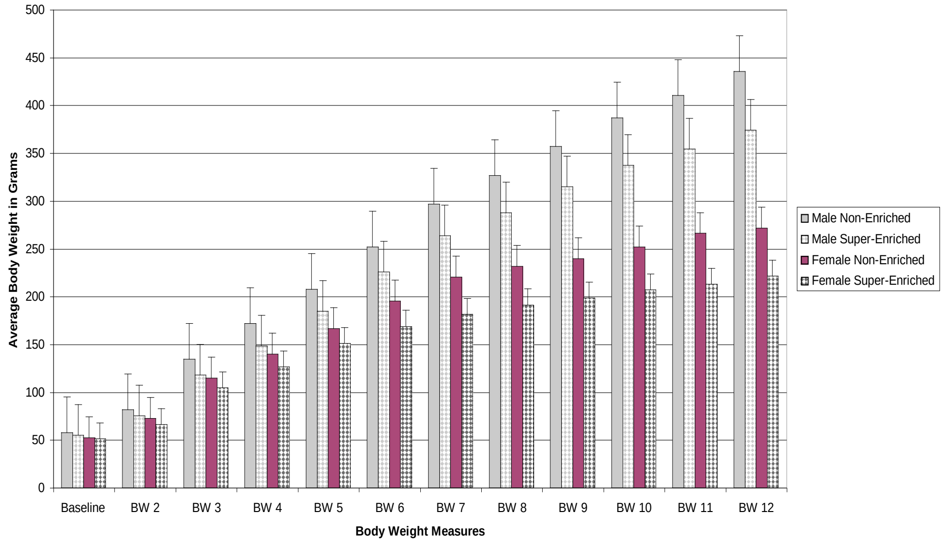


Figure 19b. Experiment III Body Weight (line)

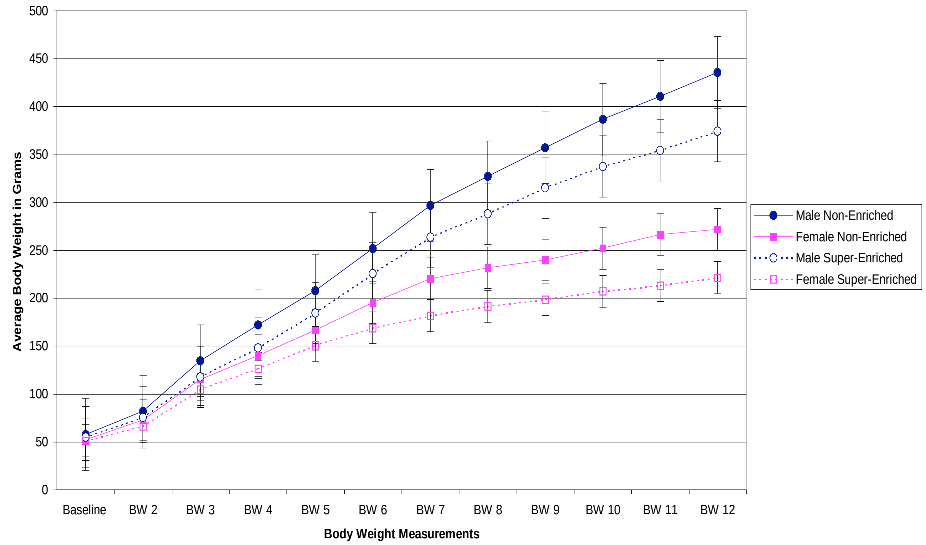


Figure 19c. Experiment III End of Experimental Phase Weight Comparison

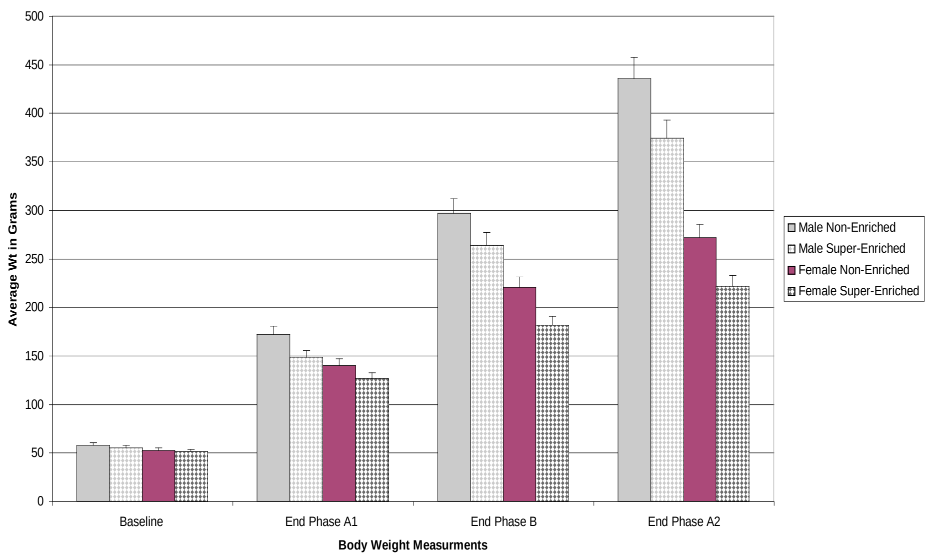


Figure 20. Experiment III BMI Index

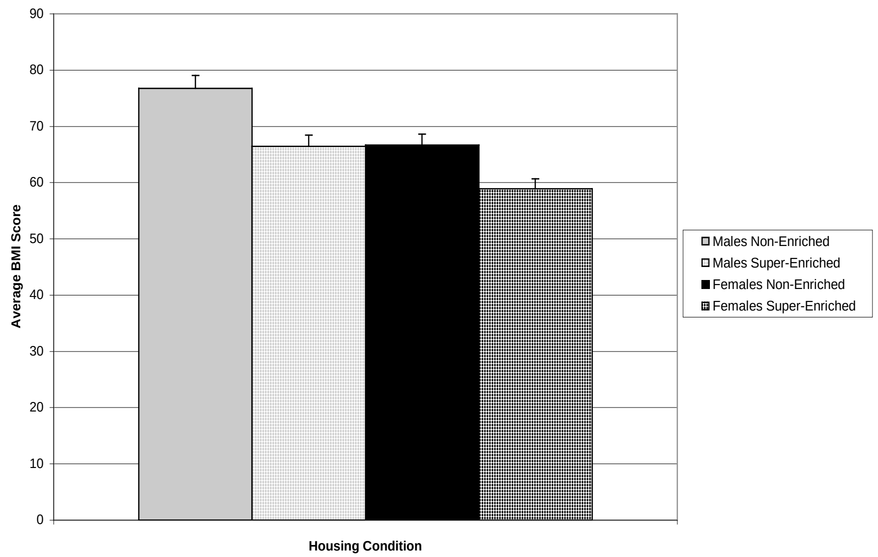


Figure 21. Experiment III Lee Index

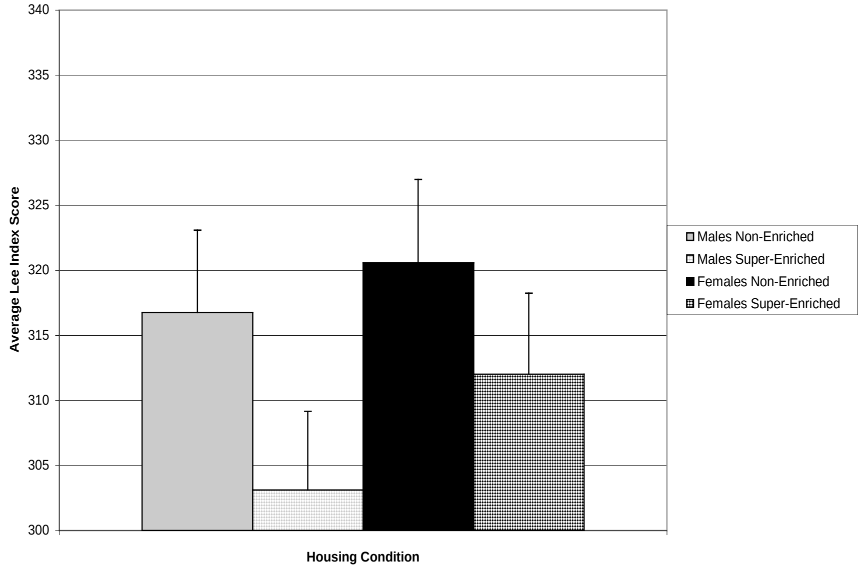


Figure 22a. Experiment III Average Number of Grams Consumed Daily by Experimental Phase

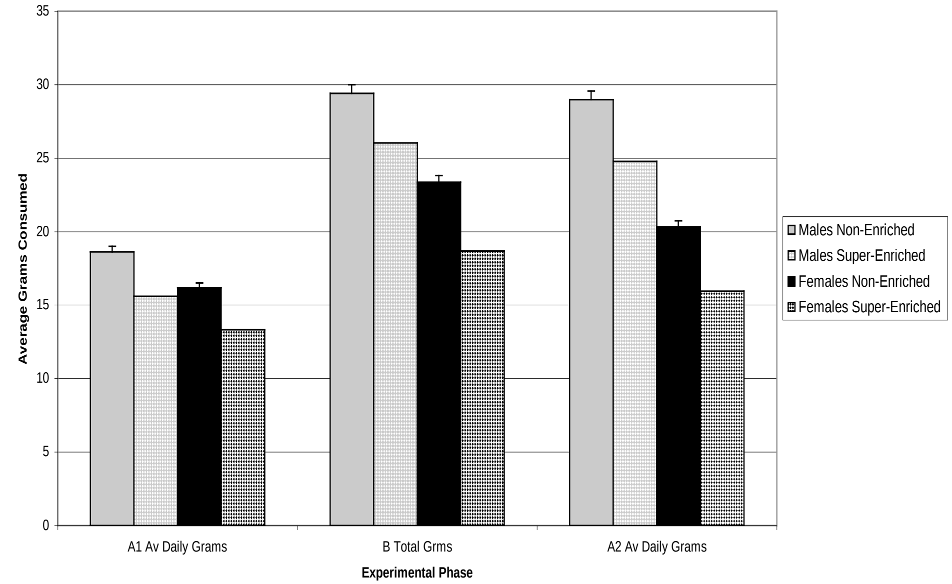


Figure 22b. Experiment III Average Number of Calories Consumed Daily by Experimental Phase

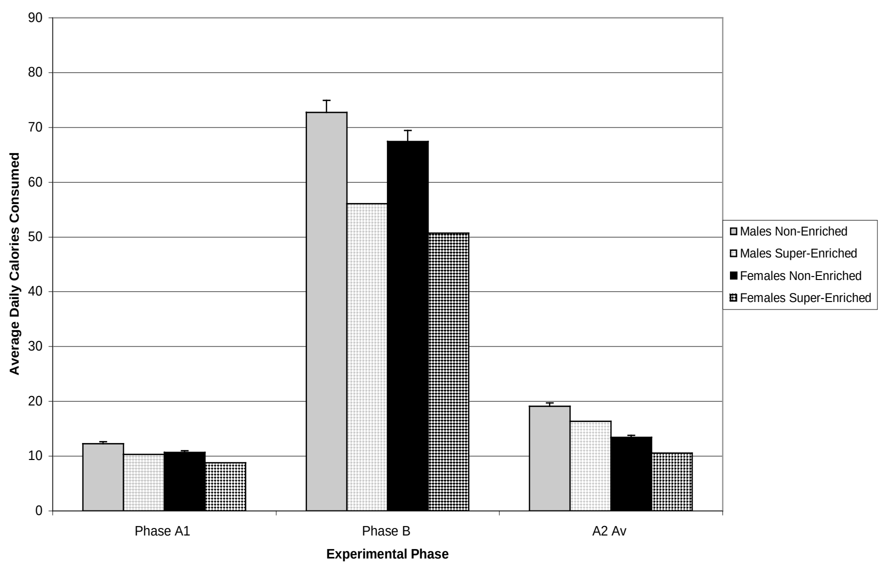


Figure 22c. Experiment III Phase B Average Grams Breakdown

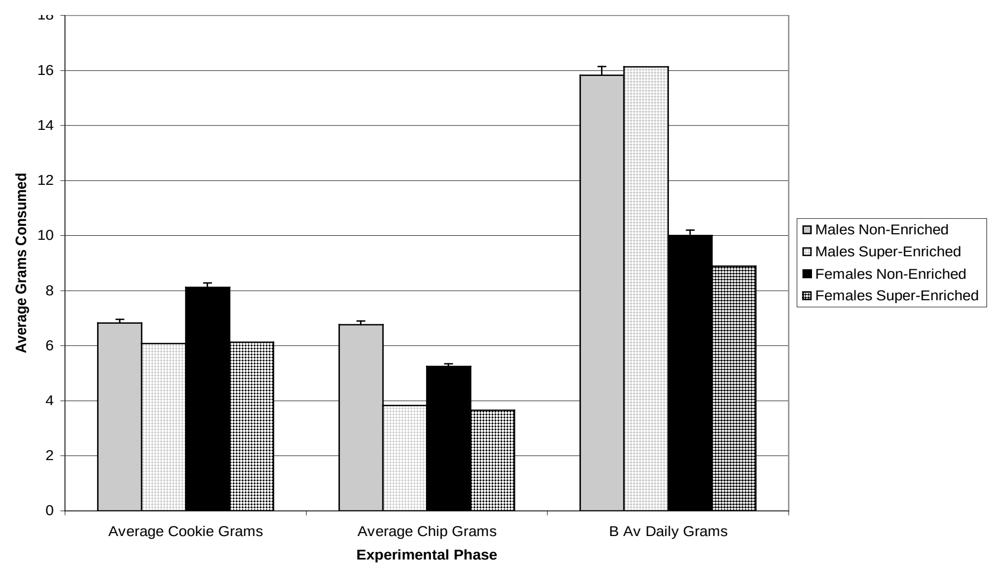


Figure 22d. Experiment III Phase B Average Calories Breakdown

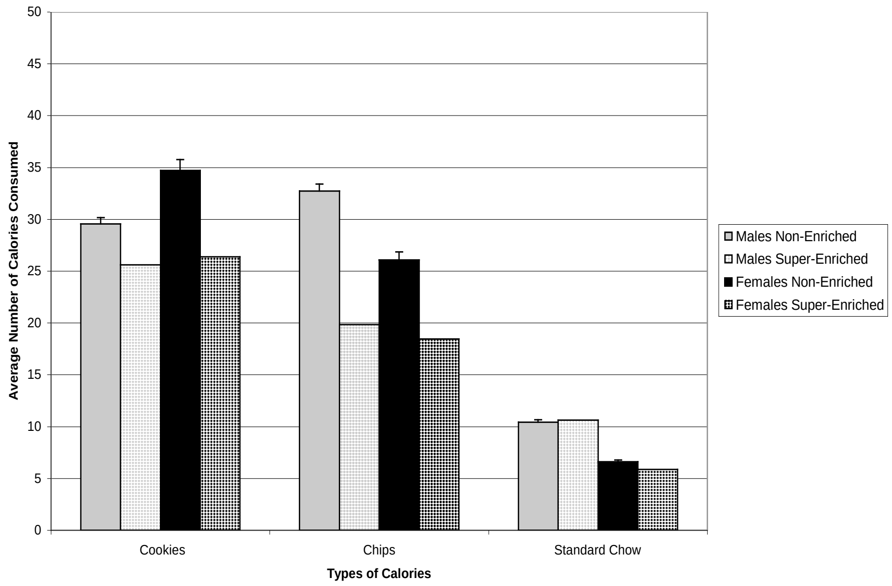


Figure 23a. Experiment III Median Score for Number of Animals Moving by Experimental Phase

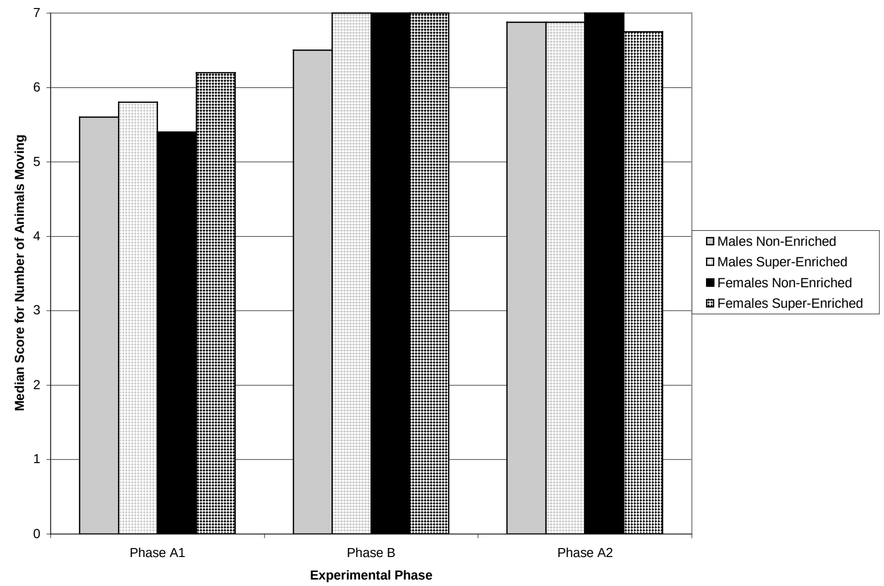


Figure 23b. Experiment III Median Score for Amount of Physical Activity by Experimental Phase

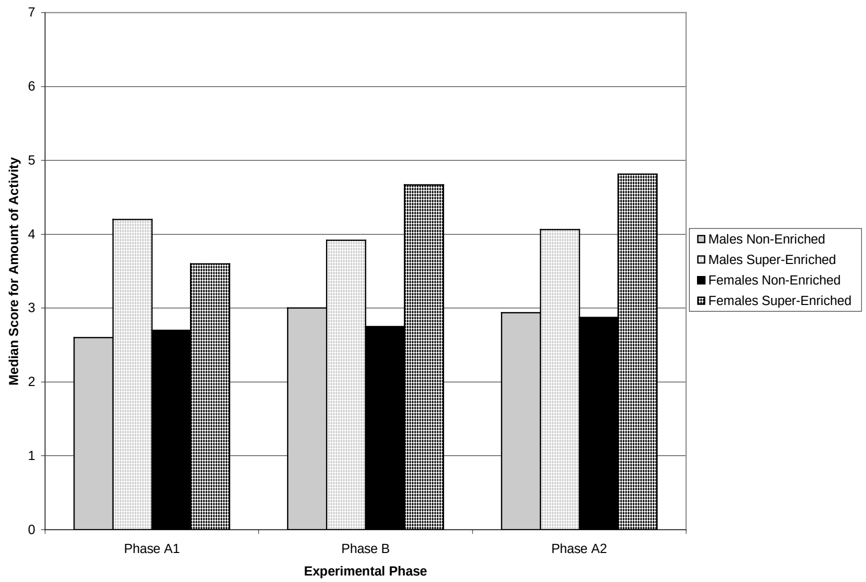
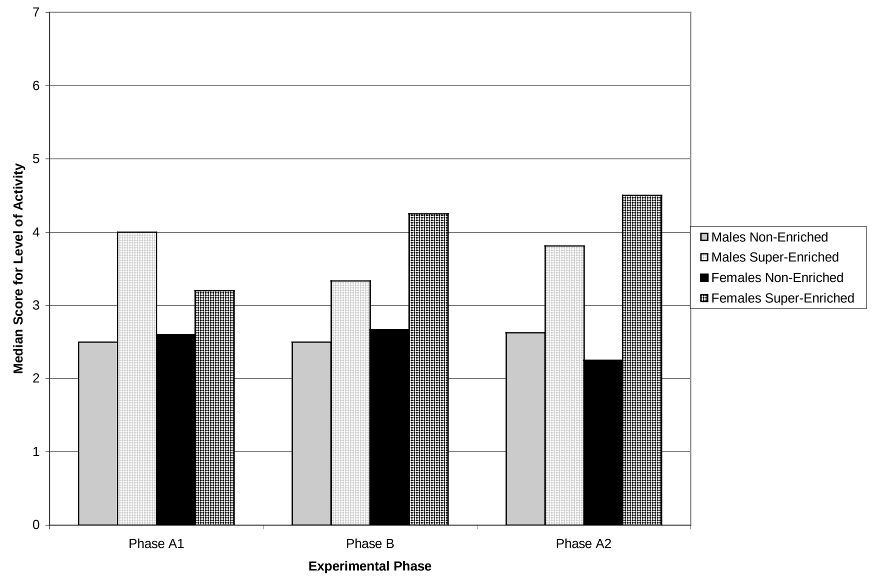
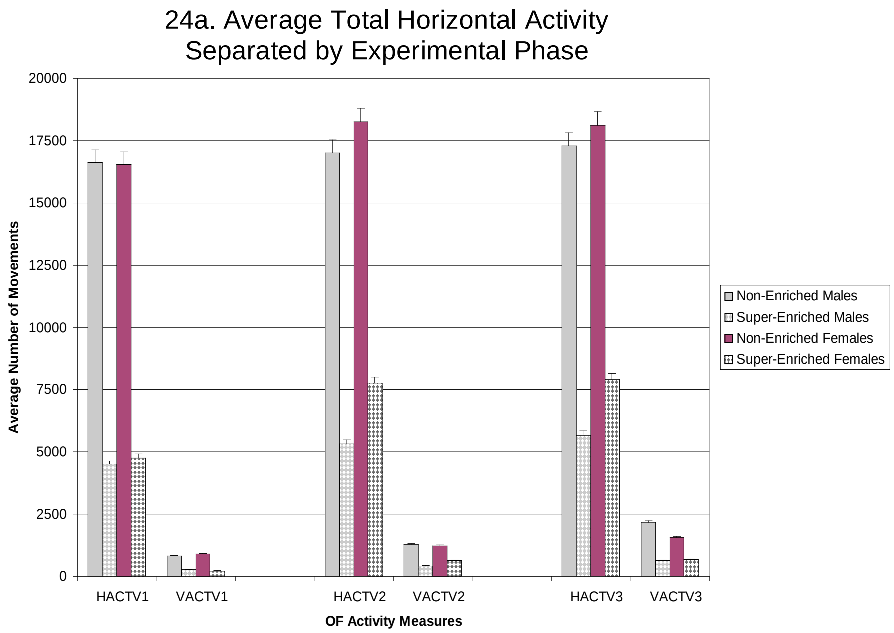
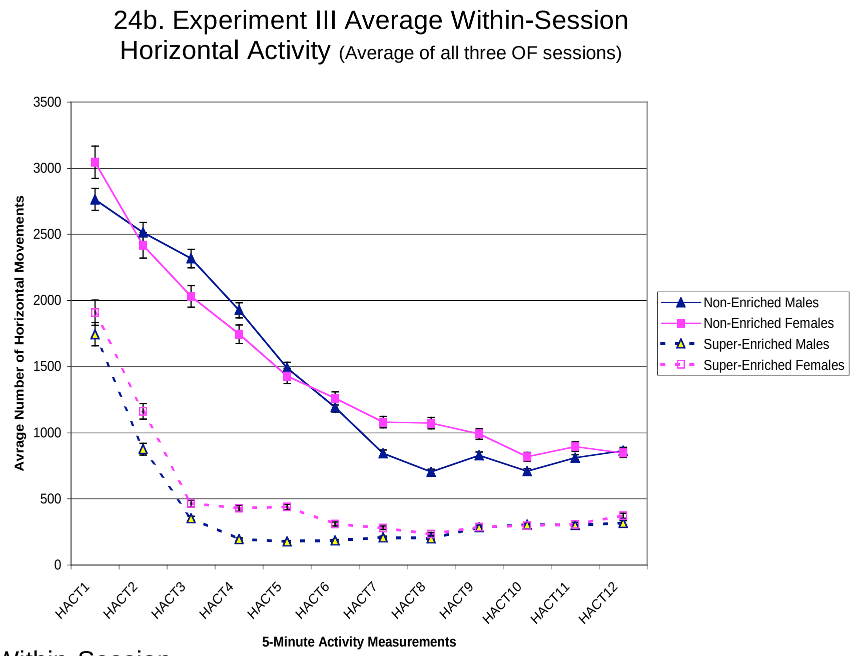


Figure 23c. Experiment III Median Score for Level of Physical Activity by Experimental Phase




24a. Average Total Horizontal Activity
Separated by Experimental Phase
Average Number of Movements
OF Activity Measures
HACTV1
VACTV1
HACTV2
VACTV2
HACTV3
VACTV3
Non-Enriched Males
Super-Enriched Males
Non-Enriched Females
Super-Enriched Females


24b. Experiment III Average Within-Session
Horizontal Activity (Average of all three OF sessions)
Avrage Number of Horizontal Movements
5-Minute Activity Measurements
Non-Enriched Males
Non-Enriched Females
Super-Enriched Males
Super-Enriched Females

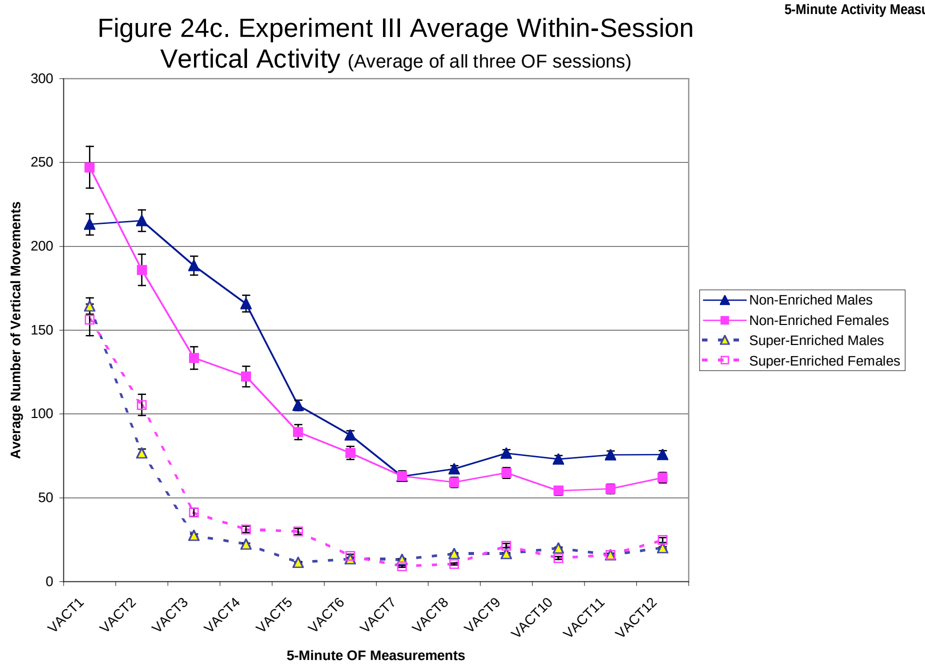


Figure 24c. Experiment III Average Within-Session
Vertical Activity (Average of all three OF sessions)
Average Number of Vertical Movements
5-Minute OF Measurements
Non-Enriched Males
Non-Enriched Females
Super-Enriched Males
Super-Enriched Females

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